

Hormonal Control of Liver Regeneration

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Two peaks in cyclic AMP production in rat livers 4 and 12h after partial hepatectomy (MacManus *et al.*, 1972) were confirmed and a third peak established at 22h, which is the peak of DNA synthesis. The increases in cyclic AMP were prevented by β -adrenergic blocking agents, propranolol and pindolol, without affecting ornithine decarboxylase induction or DNA synthesis. The α -blocking agents, phenoxybenzamine and phentolamine, given at the time of partial hepatectomy, delayed the rise in ornithine decarboxylase normally found 4h after operation, but did not affect DNA synthesis. If the α -blocking agents were given at 9–12h or 18h, the onset of DNA synthesis was delayed. Phenoxybenzamine did not affect the induction of ornithine decarboxylase in intact rat livers by glucagon or growth hormone, but did inhibit induction by dexamethasone. The induction of ornithine decarboxylase produced by dexamethasone was inhibited by 17α -hydroxyprogesterone; this compound also blocked the induction of ornithine decarboxylase in livers of partially hepatectomized rats.

Hormones of the anterior pituitary, the adrenals and the pancreas are not essential for the process of liver regeneration, but the onset of the regenerative process is facilitated in their presence (Harkness, 1957). The regeneration can be monitored biochemically by measuring the incorporation of [3 H]thymidine into DNA, which occurs between 18 and 24h after partial hepatectomy in normal rats, or by measuring the induction of ornithine decarboxylase (Russell & Snyder, 1968; Jänne & Raina, 1968) of which the first peak occurs at about 4h after partial hepatectomy. This is followed by a second peak whose timing is dependent on the age and probably the nutritional status of the rat (Hölttä & Jänne, 1972).

Early in the regeneration there is also an increase in the concentration of cyclic AMP, which is maximal about 4h after the operation; this is succeeded by a second peak at about 12h (MacManus *et al.*, 1972, 1973). Because of the involvement of cyclic AMP we decided to look at the effect of agents which interfere with the catecholamine receptors to see if these would affect the regenerative process (Ord & Stocken, 1972a; Thrower & Ord, 1972; Thrower *et al.*, 1973).

β -Blocking agents such as propranolol were ineffective in preventing the first wave of ornithine decarboxylase induction, when given at the time of partial hepatectomy. On the other hand phenoxybenzamine, which blocks α -receptors responding primarily to noradrenaline, delayed the appearance of ornithine decarboxylase. If the drug was administered at about 9h after partial hepatectomy the first wave of DNA synthesis was inhibited (Thrower *et al.*, 1973). With rats kept under controlled lighting conditions, and the time of access to food carefully restricted, it is

possible to obtain animals whose diurnal rhythms are tightly synchronized (Hopkins *et al.*, 1973). By using rats maintained under these conditions before and after partial hepatectomy it was possible to define more precisely the periods of sensitivity to phenoxybenzamine, and to confirm that the effects which are found are probably due to its α -adrenergic blocking action since they are also produced by phentolamine. Additionally, the effects of actinomycin D at different times during the first 24h of regeneration were examined.

By studying the effects of these drugs on ornithine decarboxylase induction, on the production of cyclic AMP, and on the factors associated with DNA synthesis, some insight was gained into the role of catecholamines and glucocorticoids in promoting the early stages in compensatory hyperplasia after partial hepatectomy.

Materials and Methods

Animals

Male Wistar rats from Scientific Products Ltd. (Charles River U.K. Ltd.), Manston Road, Margate, Kent, U.K., were maintained on a strict feeding and lighting schedule. All rats were fed daily between 12:00 and 15:00h. Some rats had light from 06:00 to 20:00h and were operated on between 10:00 and 13:00h. Others had light from 15:00 to 05:00h and were operated on between 19:00 and 22:00h. Rats were used when they weighed 180–200g. Partial hepatectomies were performed under ether anaesthesia by the procedure of Higgins & Anderson (1931).

Adrenalectomized rats were maintained on 0.9% (w/v) NaCl, and used after 4 days.

Administration of drugs and hormones

Reserpine was injected intraperitoneally in a vehicle containing citric acid, Tween 80 and 2-methylpropan-1-ol in water. Other drugs and hormones were injected intraperitoneally as solution or suspension in 0.9% NaCl or 60% (v/v) ethanol. The 0.9% NaCl or 60% ethanol was given similarly to control animals. Growth hormone was obtained from Calbiochem Ltd., London W1H 1AS, U.K.; cyclic AMP, glucagon, dexamethasone and noradrenaline were obtained from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey KT2 7BH, U.K. The phenoxybenzamine was a gift from Smith, Kline and French Ltd., Welwyn Garden City, Herts., U.K. Phentolamine, DL-propranolol and pindolol were gifts from Dr. E. M. Vaughan-Williams, Department of Pharmacology, South Parks Road, Oxford OX1 3QT, U.K.

Assay of ornithine decarboxylase

A 20% (w/v) homogenate of rat liver in 25mM-sodium-potassium phosphate-5mM-dithiothreitol, was centrifuged at 100000g for 1 h to provide the enzyme extract. The enzyme in the supernatant was assayed (Russell & Snyder, 1968) in the presence of 5mM-dithiothreitol; 0.1 μ mol of pyridoxal phosphate was added/ml of enzyme extract.

The first assay system contained 0.8 ml of buffer [50mM-Na₂HPO₄-KH₂PO₄ (pH 7.5)-5mM-dithiothreitol], 100 nmol of L-ornithine containing 0.1 μ Ci of DL-[1-¹⁴C]ornithine (1.6-6.7 nmol of DL-ornithine) and 1.0 ml of enzyme. The second assay system had 1.3 ml of buffer [25mM-Na₂HPO₄-KH₂PO₄ (pH 7.5)-5mM-dithiothreitol], 100 nmol of L-ornithine containing 0.1 μ Ci of DL-[1-¹⁴C]ornithine and 0.5 ml of enzyme. After a 20 min incubation at 37°C the reaction was stopped by the addition of 0.2 ml of 2.5M-H₂SO₄. The ¹⁴CO₂ produced was trapped in Hyamine 10-X (0.1 ml of 1M-Hyamine 10-X in methanol). After a further 30 min at 37°C the vial containing the Hyamine 10-X was removed and dropped into 10 ml of toluene-Triton X-100-butyl-PBD [5-(4-biphenyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole] scintillant to which 1 ml of 1M-HCl was added before counting for radioactivity. The first procedure was used only for the first two sections of Table 3, where values for partially hepatectomized rats at 3.2 and 4 h after operation were 161.4 and 157.8 c.p.m./20 min per mg of protein. Thereafter the second assay, with the decreased amount of enzyme, was followed; this gave 335 c.p.m./20 min per mg of protein for partially hepatectomized rats at 3 h after the operation (see below).

Assay of cyclic AMP

Rats were anaesthetized with ether, and the large right-hand side liver lobe remaining after 70% hepatectomy was removed and freeze-clamped. The lobe was weighed, and homogenized in 5% (w/v) trichloroacetic acid; the supernatant was frozen and stored at -20°C until assayed.

Cyclic AMP was assayed essentially by the method of Cooper *et al.* (1972). The assay was calibrated in the presence of trichloroacetic acid. Samples were purified to check the authenticity of the cyclic AMP; the measured content of cyclic AMP in the samples was unchanged after ether extraction of the trichloroacetic acid and after purification on Dowex 50 (G. Schultz, E. Böhme & J. G. Hardman, personal communication).

Preparation of histone fractions

Nuclei were isolated from rat liver by the Cemelulol detergent method (Dabeva & Tsanev, 1966), and washed in 10mM-Tris-HCl-5mM-MgCl₂, pH 7.2. Histone F1 was extracted with 5% (w/v) HClO₄ and the remaining histones were extracted with 250mM-HCl. Both fractions were precipitated with 25% (w/v) trichloroacetic acid and redissolved in water before use.

Protein determination

Protein was measured by the method of Lowry *et al.* (1951) with bovine serum albumin [Sigma (London) Chemical Co. Ltd.] as standard.

Incorporation of [³H]thymidine into DNA

Rats were given an intraperitoneal injection of 15 or 20 μ Ci of [6-³H]thymidine 1 h before death. They were killed by cervical dislocation, blood was collected and the small left-hand side lobe of the liver was removed into 5% (w/v) trichloroacetic acid, homogenized, washed three times with ethanol, washed twice at 70°C with ethanol-ether (3:1, v/v) and dried. DNA was extracted in 5% (w/v) trichloroacetic acid at 90°C for 20 min. The deoxyribose was determined by the method of Burton (1956) and the radioactivity was counted. Blood was deproteinized in 5% (w/v) trichloroacetic acid and the ³H content was measured.

Radiochemicals

All radiochemicals were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. The specific radioactivities were: [6-³H]thymidine, 30 Ci/mmol; L-[4,5(n)-³H]lysine, 8.7 Ci/mmol; DL-[1-¹⁴C]-ornithine, 15-61 mCi/mmol; [³²P]phosphate, 60-120 Ci/ μ g of P; cyclic [8-³H]AMP, 30 Ci/mmol.

Radioactivity determinations

Millipore filters used in the measurements of cyclic AMP were dissolved in 1ml of 2-methoxyethanol and counted in 10ml of toluene-2-methoxyethanol-butyl-PBD scintillant [200ml of 2-methoxyethanol+800ml of 0.5% (w/v) butyl-PBD in toluene]. Other ^3H radioactivity was counted in dioxan scintillant. The efficiency was about 30%. ^{14}C radioactivity was counted in toluene-Triton X-100-butyl-PBD [300ml of Triton X-100+700ml of 0.5% (w/v) butyl-PBD in toluene] with an efficiency of about 85%. All counts were recorded to give an accuracy of $\pm 2\%$.

Results

Sensitivity of DNA synthesis to adrenergic blocking agents and actinomycin D

Our earlier experiments had shown that although the induction of ornithine decarboxylase was delayed by giving phenoxybenzamine to partially hepatectomized rats at the time of operation, this did not affect ^3H thymidine incorporation into DNA at 22h (Thrower *et al.*, 1973). When the drug was given at 9-12h, however, DNA synthesis at 22h was diminished, although it was unaffected if the phenoxy-

benzamine was administered at 21h. More careful examination of the timing of the sensitivity of DNA synthesis at 22h to phenoxybenzamine administration indicated the precision of the response to the drug (Fig. 1). At 5-8h after partial hepatectomy the drug had no effect on DNA synthesis at 22h, but it was markedly inhibitory at 9-12h, being most effective if given at 12h. Administration at 18h also decreased ^3H thymidine incorporation into DNA at 22h.

Phenoxybenzamine is primarily an α -adrenergic blocking drug, but doubts have been raised about the specificity of its action (Furchgott, 1972). Therefore we repeated the experiments with phentolamine, which is a different class of α -adrenergic blocking agent (Fig. 1). The results confirmed the existence of the two points of sensitivity of DNA synthesis to α -adrenergic blockage.

Fujioka *et al.* (1963) studied the effect of repetitive low doses of actinomycin D given at early times in regeneration and showed that these delayed DNA synthesis. Therefore we examined the effect on the peak of DNA synthesis at 22h of a single low dose of actinomycin D ($35\mu\text{g}/\text{kg}$) given at various times during regeneration. The pattern of response to actinomycin was more complex than that to the α -adrenergic

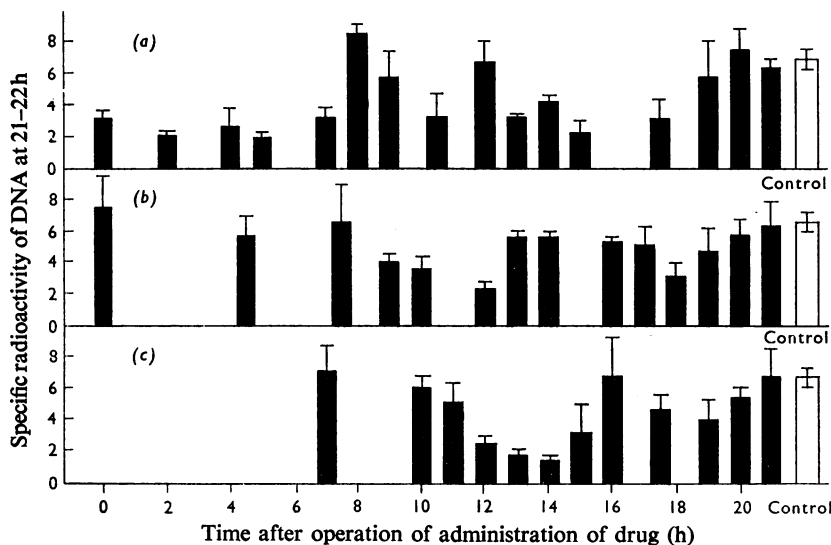


Fig. 1. Effects on the peak of DNA synthesis 21-22h after partial hepatectomy of the time of administration of α -adrenergic blocking agents and actinomycin D

Single doses of actinomycin D ($35\mu\text{g}/\text{kg}$), phenoxybenzamine ($5\text{mg}/\text{kg}$) and phentolamine ($10\text{mg}/\text{kg}$) were given intraperitoneally at the indicated times after partial hepatectomy, and the incorporation of ^3H thymidine into DNA at 21-22h was measured as described in the Materials and Methods section. Thymidine incorporation is expressed as $10^2 \times \text{c.p.m.}/\text{mg}$ of DNA relative to the ^3H c.p.m./ml of blood. The s.e.m. for each point is indicated by the bar. All the rats were kept in light from 06:00 to 20:00h and with food available *ad lib*. Groups of three to five rats were used at each time-point and experiments at 10-13h and 17-20h after partial hepatectomy were performed at least twice with each drug. (a) Actinomycin D; (b) phenoxybenzamine; (c) phentolamine.

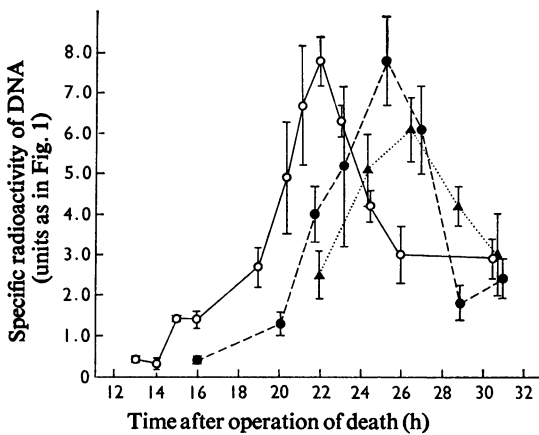


Fig. 2. Time-course of DNA synthesis in regenerating liver after administration of actinomycin D or phenoxybenzamine 9–11 h after operation

Partially hepatectomized rats were given a single dose of phenoxybenzamine (5 mg/kg body wt.) at 9 h after operation or actinomycin D (35 μ g/kg body wt.) at 11 h after operation, and the incorporation of [3 H]thymidine into DNA was determined at times between 12 and 30 h after operation as described in the Materials and Methods section. Groups of three to five rats were used for each time point. \circ , Control; \bullet , phenoxybenzamine-treated; \blacktriangle , actinomycin D-treated.

blocking agents, but the two points of sensitivity at 10.5 h and at 15–17 h resembled and preceded the time of maximal sensitivity to phenoxybenzamine and phentolamine. The effect of both actinomycin D and phenoxybenzamine, when given at 9–11 h (Fig. 2), was to delay DNA synthesis. Like phenoxybenzamine, actinomycin D given at 21 h did not inhibit DNA synthesis at 22 h (Fig. 1).

Our earlier experiments have suggested that the effects of phenoxybenzamine at 9–12 h were due to interference in the process of enzyme induction, as was its action at the time of operation. Nuclear histone F1 phosphokinase induction (Thrower *et al.*, 1973) and thymidine phosphorylation (Ord & Stocken, 1974) were delayed so that the activities of the enzymes at 18 h after partial hepatectomy were low. [32 P]Phosphate incorporation into histone F1 was similarly delayed (Table 1a), being markedly inhibited at 18.5 h. The same pattern of inhibition was observed for histone F1 biosynthesis, measured as [3 H]lysine incorporation, and for DNA synthesis, measured as [32 P]phosphate incorporation (Table 1a). Table 1(b) shows that phenoxybenzamine inhibition was not due to inhibition of general protein synthesis. Actinomycin D given at 11 h also inhibited both DNA synthesis and histone F1 biosynthesis at 21.5 h (Table 1c).

We also examined the effect of phenoxybenzamine given at the later blocking time of 18 h (Table 1d): in this case the inhibition of DNA synthesis at 22 h was

Table 1. Effect of phenoxybenzamine given at 9 and 18 h, and of actinomycin D given at 11 h, after partial hepatectomy on protein and DNA synthesis in regenerating rat liver

Actinomycin D (35 μ g/kg body wt.) was administered intraperitoneally at 11 h (c). Phenoxybenzamine (5 mg/kg body wt.) was given intraperitoneally at 9 h (a and b) or 18 h (d). Where used, [3 H]lysine (10 μ Ci/200 g rat) and [3 H]thymidine (15 μ Ci/200 g rat) were given 1 h before death and [32 P]phosphate was given 1.5 h before death. Extraction of DNA, histone and total protein was as described in the Materials and Methods section. In Expt. (a), [32 P]phosphate incorporation into DNA and histone is given as $10^6 \times$ c.p.m./ μ g of DNA or $10^6 \times$ c.p.m./ μ g of F1 histone relative to the acid-soluble 32 P c.p.m./g of liver; [3 H]lysine incorporation into histone is given as $10^3 \times$ c.p.m./ μ g of F1 histone relative to the acid-soluble 3 H c.p.m./g of liver. In Expts. (b)–(d), 3 H incorporation is expressed as [3 H]thymidine c.p.m./ μ g of DNA or [3 H]lysine c.p.m./ μ g of protein. The values given are means \pm S.E.M., with the numbers of experiments in parentheses.

Treatment	Time of death (h after operation)	DNA synthesis	F1 histone synthesis	F1 histone phosphorylation	Total protein synthesis
(a) Control	18.5	5.0 \pm 0.57 (3)	72 \pm 2.4 (3)	0.71 \pm 0.084 (3)	
	21.5	5.2 \pm 0.99 (3)	61, 34 (2)	0.55 \pm 0.054 (3)	
+Phenoxybenzamine	18.5	1.6 \pm 0.42 (3)	18 \pm 3.2 (3)	0.14 \pm 0.038 (3)	
	21.5	3.7 \pm 0.17 (4)	37 \pm 4.1 (4)	0.43 \pm 0.051 (4)	
(b) Control	18.5	2.0 \pm 0.44 (6)	2.2 \pm 0.27 (6)		0.17 \pm 0.015 (4)
	21.5	2.7 \pm 0.22 (6)	2.4 \pm 0.28 (6)		0.20 \pm 0.007 (4)
+Phenoxybenzamine	18.5	0.8 \pm 0.18 (6)	1.2 \pm 0.22 (6)		0.19 \pm 0.016 (4)
	21.5	1.1 \pm 0.50 (6)	1.7 \pm 0.28 (6)		0.21 \pm 0.002 (4)
(c) Control	22.0	2.7 \pm 0.61 (3)	3.0 \pm 0.53 (3)		
	22.0	2.2 \pm 0.29 (4)	1.6 \pm 0.20 (4)		
(d) Control	22.0	2.7 \pm 0.21 (4)	3.4 \pm 0.60 (4)		
	22.0	1.4 \pm 0.42 (3)	3.7 \pm 0.20 (3)		

not accompanied by inhibition of histone F1 biosynthesis.

Cyclic AMP changes in regenerating liver and the effect of adrenergic blocking agents

It has been suggested that changes in the cyclic AMP concentration may have a controlling effect in early regeneration (MacManus *et al.*, 1972, 1973; Gaza *et al.*, 1973). The concentrations of cyclic AMP in our partially hepatectomized rats (Fig. 3) confirmed that there were increases in the cyclic AMP concentrations at 3–4 h and at 13 h after partial hepatectomy (MacManus *et al.*, 1972, 1973), and indicated the existence of a third wave beginning at about 18 h and peaking at 21 h, coincident with the period of DNA synthesis. The greater size of the first wave of cyclic AMP in our rats, compared with the results of MacManus *et al.* (1972, 1973), probably reflects differences in the strain used and also in the lighting and feeding schedule applied. Changes in ornithine decarboxylase activity during the first 24 h of regeneration (Fig. 4) showed similar differences from other published data (Hölttä & Jänne, 1972; Gaza *et al.*, 1973), with a large peak at 4 h and smaller ones at 11–12 h and 21 h.

The three waves of cyclic AMP are initiated at the time of sensitivity to the α -adrenergic blocking drugs (Fig. 1), i.e. at about 1.5, 12–13 and 17–18 h after partial hepatectomy, as are the first and third waves of ornithine decarboxylase. The second wave of enzyme induction at 10–12 h did not correspond to any point of sensitivity to the blocking agents.

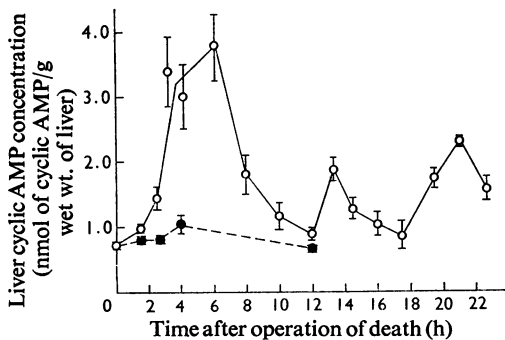


Fig. 3. Cyclic AMP concentrations in livers of rats after partial hepatectomy

Cyclic AMP concentration was measured as described in the Materials and Methods section and is expressed in nmol of cyclic AMP/g wet wt. of liver. Groups of five to ten rats were used for each time-point. \circ , Partially hepatectomized rats; \bullet , sham-operated rats.

In adrenalectomized, partially hepatectomized rats, the first wave of cyclic AMP was greatly diminished (Table 2). The β -adrenergic blocking agents, propranolol and pindolol, given at the time of operation, also inhibited the first wave of cyclic AMP (Table 2). This, with the results of MacManus *et al.* (1973), strongly suggests that catecholamines rather than glucagon are responsible for the first rise in cyclic AMP concentration after partial hepatectomy. The second wave of cyclic AMP synthesis, at 13 h, was also inhibited by pindolol, suggesting that this wave too is due to catecholamines (Table 2). Neither actinomycin D (35 μ g/kg) nor phenoxybenzamine (5 mg/kg) had an inhibitory effect on either of the first two waves of cyclic AMP synthesis (Table 2), indicating that the inhibition of ornithine decarboxylase induction by α -adrenergic blocking agents (Thrower *et al.*, 1973 and below) and of DNA synthesis by α -adrenergic blocking drugs and actinomycin D (Fig. 1) is not consequential on an inhibition of cyclic AMP production.

Phenoxybenzamine apparently increased the concentrations of cyclic AMP at 3 and 13 h in livers of partially hepatectomized rats. Rat liver behaves anomalously with respect to its α - and β -adrenergic receptors, and Exton *et al.* (1971) have data indicating that in this organ in the rat noradrenaline may stimulate adenylate cyclase. When the rats were reserpinized to deplete catecholamine concentrations, the effect of partial hepatectomy on liver cyclic AMP production at 3 h was decreased (Table 2), and phenoxybenzamine no longer enhanced the response of partially hepatectomized rats.

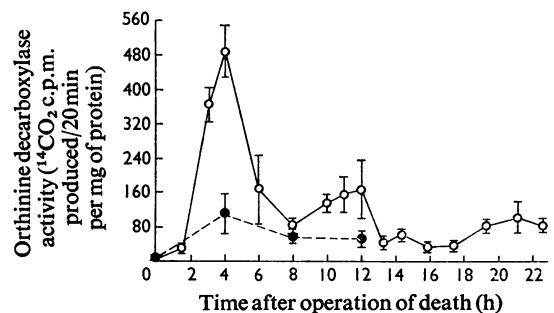


Fig. 4. Changes in ornithine decarboxylase activity during liver regeneration

Enzyme activity was measured as described in the Materials and Methods section. Ornithine decarboxylase activity is expressed as $^{14}\text{CO}_2$ c.p.m. produced/20 min per mg of protein. Groups of five to ten rats were used for each time-point. \circ , Partially hepatectomized rats; \bullet , sham-operated rats.

Table 2. Cyclic AMP concentrations in livers of partially hepatectomized rats: the effects of adrenalectomy, adrenergic blocking agents, treatment with reserpine and actinomycin D

Rats were adrenalectomized 4 days before use. Reserpinized rats were given 1 mg of reserpine/kg at 72, 48 and 24h before operation. All drugs were given intraperitoneally. The doses were: actinomycin D, 35 µg/kg body wt.; phenoxybenzamine, 5mg/kg; propranolol, 10mg/kg; pindolol, (1) 0.5mg/kg, (2) 1.0mg/kg. Cyclic AMP concentrations are expressed as nmol of cyclic AMP/g wet wt. of liver ±S.E.M., with the numbers of animals in parentheses.

Treatment	Time after operation (h)		Cyclic AMP concentration
	Treatment	Death	
Sham-operated	—	4	1.04 ± 0.13 (4)
Partial hepatectomy	—	4	2.94 ± 0.56 (4)
Adrenalectomy + partial hepatectomy	—	4	1.65 ± 0.08 (3)
+Phenoxybenzamine	-0.5	4	2.97 ± 0.51 (3)
Partial hepatectomy	—	3	3.37 ± 0.53 (5)
Reserpinized partially hepatectomized	—	3	1.90 ± 0.65 (4)
+Phenoxybenzamine	-0.5	3	1.05 ± 0.30 (3)
Partial hepatectomy	—	2.5	1.83 ± 0.29 (11)
+Phenoxybenzamine	-0.5	2.5	3.26 ± 0.79 (5)
+Actinomycin D	-0.5	2.5	2.52 ± 0.41 (6)
+Propranolol	-0.5	2.5	0.85 ± 0.33 (4)
Partial hepatectomy	—	3	3.47 ± 0.51 (9)
+Pindolol (1)	+1.0	3	2.58 ± 0.29 (6)
+Pindolol (2)	+1.2	3	1.96 ± 0.28 (5)
Partial hepatectomy	—	13	2.01 ± 0.17 (9)
+Phenoxybenzamine	+10	13	2.58 ± 0.31 (4)
+Pindolol (2)	+12	13	1.54 ± 0.08 (4)
Partial hepatectomy	—	12.8	1.68 ± 0.42 (4)
+Actinomycin D	+10.5	12.8	1.62 ± 0.31 (4)

Effects of adrenergic blockers on the induction of ornithine decarboxylase in normal and regenerating livers

The induction of ornithine decarboxylase is another early event in liver regeneration (Russell & Snyder, 1968; Jänne & Raina, 1968). Phenoxybenzamine (5mg/kg) given just before partial hepatectomy delays the induction of the enzyme (Thrower & Ord, 1972), which begins at 1.5h after operation (Fig. 4). If the administration of the drug was delayed until 1h after partial hepatectomy its inhibitory effects were still evident (Table 3), but by 2h it had little effect on the amount of enzyme at 4h. The more selective α -blocking agent, phentolamine, behaved similarly (Table 3). Propranolol and pindolol, at doses which decreased the first wave of cyclic AMP synthesis (Table 2), did not decrease the activity of ornithine decarboxylase (Table 3), indicating that elevated concentrations of cyclic AMP were not obligatory for the induction process.

The amount of ornithine used in the assay (Russell & Snyder, 1968) gives concentrations below saturation for ornithine decarboxylase, but if 2mM-L-ornithine is used, which is about ten times K_m (Raina & Jänne, 1968) the effect of partial hepatectomy is unchanged (Hölttä & Jänne, 1972). The intracellular concentration of ornithine in the liver is increased 2–3-fold by 1h after partial hepatectomy (Ord &

Stocken, 1972b). This rise in the amount of unlabelled ornithine added to an assay system will affect the apparent increase in ornithine decarboxylase concentrations after partial hepatectomy reported here. Phenoxybenzamine does not affect (G. Hughes, personal communication) the entry of α -aminoisobutyric acid into livers 1h after partial hepatectomy nor the increase (I. Lieberman, personal communication) in α -aminoisobutyric acid entry detectable by 3h after operation. Uptake of [3 H]lysine into total liver proteins (Table 1b) was also unaffected by phenoxybenzamine, suggesting that the drug affected neither the uptake of amino acids into the liver nor the intracellular amino acid pool. The decreased ornithine decarboxylase activity after phenoxybenzamine administration is therefore unlikely to be due to alterations in intracellular ornithine concentrations.

In adrenalectomized, partially hepatectomized rats, amounts of ornithine decarboxylase at 4 and 6h were very low (Table 3), so that induction of the enzyme in regenerating livers did not seem to be significantly promoted by glucagon in the absence of glucocorticoids. Glucagon can be used to induce ornithine decarboxylase in intact livers (Panko & Kenney, 1971); if phenoxybenzamine was given 0.5h before the hormone, at doses three times those used in the partially hepatectomized rats, the drug had no effect on the induction process (Table 4). Similarly

Table 3. *Inhibition of ornithine decarboxylase induction after partial hepatectomy*

Reserpined rats received three doses of 1 mg of reserpine/kg at 72, 48 and 24h before operation. Other drugs were given intraperitoneally as shown. Ornithine decarboxylase was assayed as described in the Materials and Methods section. Ornithine decarboxylase activity is expressed as $^{14}\text{CO}_2$ c.p.m. released/20min per mg of protein \pm s.e.m. The number of rats used for each value is given in parentheses.

Treatment	Dose (mg/kg body wt.)	Time of administration (h after partial hepatectomy)	Time of death after partial hepatectomy (h)	Ornithine decarboxylase activity
Sham-operated	—	—	4	32.4 \pm 3.2 (3)
Partial hepatectomy	—	—	4	157.8 \pm 16.8 (9)
+Phenoxybenzamine	5	-0.5	4	47.0 \pm 7.2 (5)
+Phenoxybenzamine	5	+1.0	4	39.6 \pm 9.0 (7)
+Phenoxybenzamine	5	+2.0	4	118.0 \pm 8.4 (3)
+Phentolamine	10	-0.5	4	80.6 \pm 16.8 (3)
+Phentolamine	5	+1.2	4	78.4 \pm 23.8 (5)
Partial hepatectomy	—	—	3.2	161.4 \pm 14.8 (13)
+17 α -Hydroxyprogesterone	20	-0.5	3.2	70.6 \pm 11.6 (3)
+17 α -Hydroxyprogesterone	2 \times 10	+1.0, +1.7	3.2	75.0 \pm 10.4 (4)
+Noradrenaline	2 \times 0.5	-0.5, +1.0	3.2	309.8 \pm 34.0 (4)
+Propranolol	10	-0.5	3.2	183.2 \pm 37.0 (5)
+Actinomycin D	0.035	-0.5	3.2	242.0 \pm 50.0 (5)
Partial hepatectomy	—	—	3	335.0 \pm 58.2 (4)
+Pindolol	0.5	+1.0	3	467.4 \pm 93.4 (6)
Partial hepatectomy	—	—	3	362.0 \pm 38.0 (4)
Reserpined	3 \times 1	—	3	130.0 \pm 43.2 (4)
Reserpined + phenoxybenzamine	5	-0.5	3	104.2 \pm 9.4 (3)
Partial hepatectomy	—	—	4	595.0 \pm 69.2 (4)
Partial hepatectomy	—	—	6	265.9 \pm 79.4 (4)
Adrenalectomized	—	—	4	58.2 \pm 26.2 (3)
Adrenalectomized	—	—	6	38.8 \pm 14.4 (3)

Table 4. *Effect of α -adrenergic blocking agents and 17 α -hydroxyprogesterone on hormonal induction of ornithine decarboxylase in normal rats*

Single doses of hormone were injected intraperitoneally: glucagon 3 mg/kg; growth hormone 0.4 mg/kg; dexamethasone (1) 0.1 mg/kg, (2) 0.5 mg/kg. All inhibitors were given intraperitoneally 30min before the administration of the hormone except (3), when two doses of 25mg of 17 α -hydroxyprogesterone /kg were given, at -0.5h and at 1.0h. Ornithine decarboxylase activity was assayed as described in the Materials and Methods section; its activity is expressed as $^{14}\text{CO}_2$ c.p.m. released/20min per mg of protein, \pm s.e.m. The number of rats used for each point is given in parentheses.

Treatment	Time of death after operation (h)	Ornithine decarboxylase activity
0.9% NaCl	2.6	12.4 \pm 1.2 (9)
Phenoxybenzamine (5mg/kg)	2.6	17.8 \pm 3.8 (5)
Glucagon	2.7	142.8 \pm 53.8 (5)
+Phenoxybenzamine (15mg/kg)	2.7	138.6 \pm 36.4 (5)
Growth hormone	3.1	44.0 \pm 4.6 (4)
+Phenoxybenzamine (15mg/kg)	3.1	50.4 \pm 16.0 (4)
Dexamethasone (1)	2.6	178.2 \pm 22.8 (4)
+Phenoxybenzamine (15mg/kg)	2.6	71.8 \pm 19.2 (8)
Dexamethasone (2)	2.6	404.4 \pm 64.8 (8)
+Phenoxybenzamine (10mg/kg)	2.6	283.6 \pm 84.8 (4)
+Phentolamine (30mg/kg)	2.6	189.0 \pm 49.8 (4)
Dexamethasone (2)	2.6	622.0 \pm 109.4 (4)
+17 α -Hydroxyprogesterone (1 \times 50mg/kg)	2.6	422.0 \pm 37.6 (4)
+17 α -Hydroxyprogesterone (2 \times 25mg/kg) (3)	2.6	280.4 \pm 61.6 (4)

it did not affect (Table 4) the induction of ornithine decarboxylase by growth hormone (Russell & Snyder, 1969).

Reserpinized partially hepatectomized rats showed a lower induction of enzyme (Table 3) than normal partially hepatectomized rats; conversely, noradrenaline administration caused a twofold increase in the amount of ornithine decarboxylase present 4h after partial hepatectomy (Table 3).

Glucocorticoids can also induce ornithine decarboxylase (Panko & Kenney, 1971). The induction produced by dexamethasone was markedly decreased by phenoxybenzamine and phentolamine (Table 4).

17 α -Hydroxyprogesterone has been shown to block cortisol and dexamethasone binding to steroid-receptor sites in rat liver (Baxter & Tomkins, 1970). In normal rats 17 α -hydroxyprogesterone inhibited dexamethasone induction of ornithine decarboxylase (Table 4); the drug also inhibited ornithine decarboxylase induction after partial hepatectomy (Table 3). This evidence strongly implicates glucocorticoids in the induction of ornithine decarboxylase in liver regeneration.

Discussion

Hormones from the anterior pituitary or adrenals are not obligatory for the compensatory hyperplasia of partially hepatectomized rats (Harkness, 1957), but in their absence the process of regeneration is delayed. The experiments presented here suggest that catecholamines are promoting the activation of adenylate cyclase 2–4h after operation, and that glucocorticoids and possibly noradrenaline are affecting the induction of ornithine decarboxylase at the same time. Glucagon does not appear to be involved in either process.

Both cyclic AMP and glucocorticoids induce ornithine decarboxylase in normal rats (Beck *et al.*, 1972), but their mechanisms of action are unknown, although Fausto (1971) has shown that in adrenalectomized rats amino acids or their metabolites can promote ornithine decarboxylase induction.

Our results show that in partially hepatectomized rats ornithine decarboxylase is still induced when adenylate cyclase stimulation has been prevented by propranolol or pindolol. Conversely, enzyme induction is prevented by α -adrenergic blocking agents at doses which do not affect cyclic AMP concentrations. These data taken together strongly suggest that cyclic AMP is not obligatorily required in the early induction of ornithine decarboxylase.

MacManus *et al.* (1973) have shown that the first wave of cyclic AMP production after partial hepatectomy is not essential for DNA synthesis. With 30mg of phenoxybenzamine/kg or 30mg of DL-propranolol/kg given at 8h after partial hepatectomy they obtained a delay in the second peak of cyclic AMP, and in DNA synthesis. From this they con-

cluded that this wave of cyclic AMP was essential for DNA synthesis to take place. With the smaller doses of phenoxybenzamine used here, although DNA synthesis was still inhibited, the second wave of cyclic AMP synthesis was unchanged. Conversely, 1.0mg of pindolol/kg at 12h inhibited the rise in cyclic AMP concentrations at 13h (Table 2), without affecting DNA synthesis, and even with doses of up to 5mg/kg, DNA synthesis at 22h was unaffected [thymidine uptake into DNA of untreated partially hepatectomized rats $6.9 \pm 0.5(5)$; partially hepatectomized rats given 5mg of pindolol/kg at 11.5h, $6.9 \pm 0.8(6)$]. It thus seems that neither of the two early peaks in cyclic AMP concentrations is essential for DNA synthesis after partial hepatectomy.

Whatever their function, however, the peaks in cyclic AMP production coincide with periods of sensitivity of the regenerative process to α -adrenergic blocking drugs, and with the initiation of waves of enzyme induction (see Bucher & Malt, 1971; Bresnick, 1971). Ornithine decarboxylase increases coincidentally with the first wave of cyclic AMP; the second cyclic AMP wave at 12–13h is at the time when signals are given for induction of enzymes necessary for DNA replication. At the time of the third wave, DNA synthesis and the synthesis of enzymes involved in replication are accelerating sharply (Fig. 2). The importance of these periods of protein synthesis is underlined by the effects of single low doses of actinomycin D on the peak of DNA synthesis. This is delayed by actinomycin given up to 7h after operation, or at 10.5h, or between 15 and 17h, indicating that RNA synthesis is essential at these times.

The sites for α -adrenergic blocking agents and the mechanism of action of noradrenaline on rat liver are uncertain, as is the relation between the effects of noradrenaline and glucocorticoids. Exton *et al.* (1972) have discussed the role of glucocorticoids in liver in maintaining responsiveness to cyclic AMP. The sensitivity of liver regeneration to phenoxybenzamine, phentolamine and 17 α -hydroxyprogesterone suggests that in rats noradrenaline and glucocorticoids may be implicated in the inducible protein synthesis after operation, but the factors leading to the periodicity in cyclic AMP concentrations and in protein induction are not yet understood. The greater sensitivity of inducible rather than endogenous protein synthesis to the actions of the α -adrenergic blockers parallels the effects of ionizing radiation (Van Lancker, 1970) on liver regeneration.

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