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EFFECTS OF PHENOXYBENZAMINE ON EARLY STAGES OF LIVER REGENERATION IN PARTIALLY HEPATECTOMIZED RATS

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Abstract—The changes in regenerating rat liver during the first 24 hr after 70 per cent hepatectomy have been studied using phenoxybenzamine, an adrenergic blocker. At 5 mg/kg, given before operation, the drug did not alter the increased uptake of ^{3}H orotic acid into the acid-soluble intracellular fraction of the liver detected at 1 hr after hepatectomy, but the increase in ^{3}H orotic acid incorporation into nuclear RNA at this time was only 40 per cent of that in untreated partially hepatectomized rats. Induction of ornithine decarboxylase (EC 4.1.1.17) was delayed by about 2 hr, but DNA synthesis was unaffected.

When 5 mg/kg phenoxybenzamine was given between 5 and 10 hr after partial hepatectomy, DNA synthesis was altered. Given 9 hr after operation the drug delayed the onset of peak DNA synthesis by 3 hr, from 21·5 to 24·5 hr. The induction of nuclear histone phosphokinase was also delayed.

AN EARLY event in liver regeneration is the appearance of ornithine decarboxylase (EC 4.1.1.17)[1,2] which is easily detectable after 2 hr and is increased more than 10-fold by 4 hr. The induction is blocked if actinomycin is given immediately after operation but not if administration of the inhibitor is delayed for 30 min.[3] The enzyme can also be induced in intact livers from normal[4] or adrenalectomized[5,6] rats by growth hormone, hydrocortisone, glucagon and other hormones.[6] The induction in regenerating rat liver was not prevented by hypophysectomy or adrenalectomy.[7]

The rapidity of the induction following partial hepatectomy suggested that it might be promoted *inter alia* by cyclic AMP similarly to the effect of this nucleotide on tyrosine transaminase (EC 2.6.1.5) induction in foetal liver explants[8] and in adult liver.[9] To explore this possibility the effects of compounds blocking α or β adrenergic receptor sites were investigated. α-Sites are preferentially affected by noradrenaline and are inhibited by phenoxybenzamine, while β sites respond to adrenaline and are blocked by propranolol. Preliminary experiments[10] showed that the induction of ornithine decarboxylase in our partially hepatectomized rats was delayed by adrenalectomy and by phenoxybenzamine at doses above 1 mg/kg but was unaffected by propranolol (4 mg/kg). Phenoxybenzamine given just before operation also reduced DNA synthesis at 22 hr after partial hepatectomy but caused no long-term reduction in the capacity of the liver to regenerate. The interference produced by phenoxybenzamine in enzyme induction and DNA synthesis in regenerating rat liver has now been further investigated.

EXPERIMENTAL

Animals. Male rats (body wt 180–200 g) of the laboratory's Wistar strain were used

and were kept under controlled lighting (0600–2000 hr) conditions. Adrenalectomized rats received 0·9% NaCl to drink.

Enzyme assays. Ornithine decarboxylase was extracted from a 35% (w/v) homogenate of rat liver in 0·05 M sodium phosphate buffer by centrifugation at 100,000 *g* for 30 min. The enzyme was stabilized throughout the extraction and assay with 5 mM dithiothreitol. $^{14}CO_2$ produced by the assay[2] was trapped in hyamine hydroxide and counted in Triton–toluene butyl BPD scintillant.

To assay nuclear histone kinase three rat livers were pooled and a nuclear suspension prepared[11] and spun at 25,000 *g* for 20 min. The supernatant was assayed for histone kinase activity. The assay mixture, total volume 0·2 ml, contained 1·32 μmole of $MgCl_2$, 13·2 μmole of Tris–HCl, pH 7·4, 0·2 μmole of γ^{32}P ATP, specific activity 50 μCi/μmole, 0·8 mg of sheep thymus histone F1. The reaction was started by the addition of 50 μl of enzyme extract (20–40 μg protein), incubated for 20 min at 37°, and stopped with 10 μl of 5 N HCl. Fifty μl of the mixture was spotted onto Whatman No. 3 filter paper and washed 6 times with 25% trichloroacetic acid. ^{32}P incorporation was measured as Cerenkov radiation in a Beckman CPM 2000 scintillation counter.

Precursor uptakes. 5 μCi of orotic acid 5-H3 (20 Ci/mmole) or 10 μCi of thymidine 6-H3 (23·3 Ci/mmole)/100 g body wt were injected intramuscularly 0·5 hr before death. When uptake into the intracellular acid-soluble supernatant from liver was examined, 2·5 μCi of inulin carboxylic acid—C14, 1·4 mCi/mmole mol. wt 5175 $\pm$ 95, were simultaneously injected and plasma collected at death. Otherwise blood was collected, deproteinized with 10% trichloroacetic acid and the radioactivity of the supernatant determined. The small left-hand liver lobe was weighed and deproteinized. From the radioactivity of the acid-soluble supernatant and the plasma, the intracellular acid-soluble ^{3}H radioactivity/g liver could be calculated. The insoluble residue was used for determinations of the specific activity of the DNA.[12] When incorporation into RNA was followed, liver nuclei were obtained by a rapid detergent method[13] before measuring the specific activity of the total nuclear RNA.[12]

RESULTS

In confirmation of our earlier findings[10] 5 mg/kg of phenoxybenzamine given immediately before partial hepatectomy considerably reduced the amount of ornithine decarboxylase detectable in the regenerating livers 4 hr after operation. A study of the time course through the first 14 hr (Fig. 1) indicated that the peak in enzyme activity was reached about 2 hr later than in untreated rats, but that by 10 hr enzyme levels were indistinguishable from those in control animals.

As might be expected from the transience of its action, this amount of phenoxybenzamine given immediately before operation did not affect DNA synthesis examined at 22 hr, but if the drug was given 9 hr after partial hepatectomy, when the wave of ornithine decarboxylase induction had passed its peak, ^{3}H thymidine incorporated into DNA was reduced. The time course (Fig. 2) again showed a delay in the peak of DNA synthesis of about 3 hr. If the compound was given simultaneously with the ^{3}H thymidine no effects were detected, indicating that the drug was not directly inhibitory to enzymes involved in DNA synthesis.

A number of enzymes associated with DNA replication are induced and appear in regenerating liver 12–15 hr after operation. These include histone phosphokinase.[11,14] To determine if phenoxybenzamine was affecting the induction of this set of enzymes,

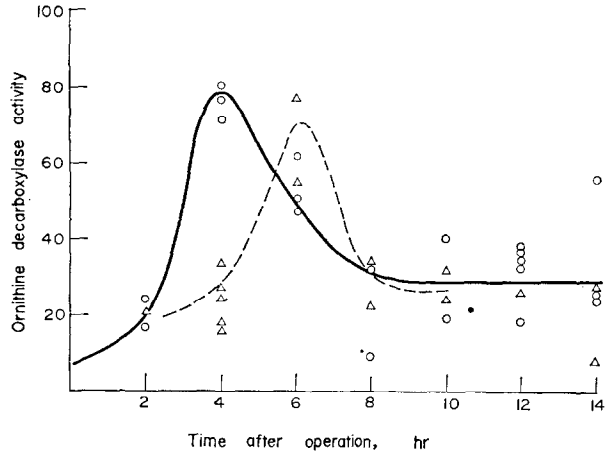

FIG. 1. Ornithine decarboxylase induction after partial hepatectomy effect of 5 mg/kg phenoxybenzamine given before operation. Phenoxybenzamine treated rats were given an intraperitoneal dose of 5 mg/kg 15 min before operation. Ornithine decarboxylase levels were determined through the first 14 hr of regeneration as described in the text. ○——○ Normal regenerating liver. △——△ Phenoxybenzamine-treated regenerating liver. Ornithine decarboxylase activity is given as pmole $^{14}CO_2$ produced/mg soluble protein/20 min.

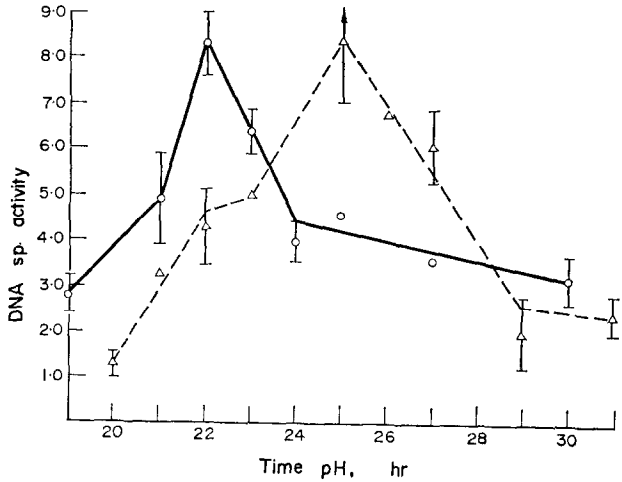

FIG. 2. Effects of 5 mg/kg phenoxybenzamine given 9 hr after partial hepatectomy on incorporation of ^3H thymidine into DNA in regenerating liver. Phenoxybenzamine-treated rats were given an intraperitoneal dose of 5 mg/kg 9 hr after operation. All rats were given 12·5 μCi 6-^3H thymidine/200 g body wt intraperitoneally 1 hr before killing. DNA was isolated as described under Experimental and the specific activity determined. ○——○ Normal regenerating liver. △——△ Phenoxybenzamine-treated regenerating liver. ^3H incorporation is expressed relative to the ^3H radioactivity of the blood (12–18,000 counts/min/ml).

levels of histone phosphokinase were examined in untreated, partially hepatectomized rats and in those which had received 5 mg/kg phenoxybenzamine at 9 hr (Table 1).

As reported previously[11] the activity of histone phosphokinase in liver nuclei increased after partial hepatectomy. The nuclear enzyme is released from chromatin for assay by reducing the osmolarity and is not activated by cyclic AMP. This observation extends our earlier results[11] and is in contrast with the histone phosphokinase

TABLE 1. LIVER NUCLEAR HISTONE PHOSPHOKINASE ACTIVITY FOLLOWING ADMINISTRATION OF 5 mg/kg PHENOXYBENZAMINE 9 hr AFTER PARTIAL HEPATECTOMY

	Untreated rats	Phenoxybenzamine treated rats
Control	10·8 ± 1·6	8·5, 11·5
Partially hepatectomized:		
13 hr	13·0	
16·5 hr	24·4, 19·2	
18 hr	32·0, 25·0	11·1, 11·5
20 hr	26·1, 27·9	19·3, 28·7

Nuclei from 2 or 3 rats were pooled for each determination. Activity is expressed as nmole phosphate incorporated into F1 histone per mg soluble protein. (1 g liver gave approx. 100 μg.)

in the cytosol studied by Langan.[15] In phenoxybenzamine-treated rats the amount of enzyme at 18 hr was not different from unoperated rats nor from livers of rats 13 hr after partial hepatectomy. However by 20 hr, when levels in partially hepatectomized rats were declining, the amount of enzyme in the livers of blocked rats was beginning to increase.

TABLE 2. ^{3}H OROTIC ACID UPTAKE INTO ACID-SOLUBLE COMPOUNDS IN RAT LIVER 1 hr AFTER PARTIAL HEPATECTOMY

	Ratio ^{3}H radioactivity Liver:plasma	Specific activity RNA
Sham-operated rats*	5·9 ± 1·2 (37)	1·0 ± 0·20 (5)
Partially hepatectomized rats	9·6 ± 2·8 (13)	2·3 ± 0·32 (4)
Adrenalectomized, partially hepatectomized rats	8·4 ± 0·8 (3)	1·4†
Phenoxybenzamine treated, partially hepatectomized rats	10·6 ± 2·1 (6)	1·4 ± 0 (3)

The ratio is given of the intracellular acid-soluble ^{3}H radioactivity in liver to the acid-soluble ^{3}H radioactivity of plasma ±S.E. The number of animals is shown in parentheses. The specific activity of total nuclear RNA is also given, relative to that in sham-operated rats. Five mg/kg phenooxybenzamine was given intramuscularly 15 min before operation. Plasma radioactivities were 40–60,000 counts/min/ml.

* In unoperated animals no differences were observed in liver:plasma ratios between normal, adrenalectomized or phenoxybenzamine-treated rats.

† Nuclei from three animals pooled.

Enzyme induction after partial hepatectomy requires the transcription of new mRNA; increased precursor uptake into total nuclear RNA is well established 4 hr after operation.[16] At earlier times interpretation is complicated by an increase in UTP pool[17] and by increased uptake from the extracellular fluid of pyrimidine compounds[18] which may be scavenged and re-utilized. Adrenalectomized rats show a slightly lower uptake of ^{3}H orotic acid into the acid-soluble components in the liver as compared with control, partially hepatectomized rats 1 hr after operation (Table 2) and only a 40 per cent increase in incorporation of the precursor into total nuclear RNA. The uptake of ^{3}H orotic acid was therefore examined in rats given phenoxybenzamine just before partial hepatectomy. The drug did not prevent the increased uptake of the pyrimidine but it did lower the incorporation into nuclear RNA (Table 2) to the same extent as in adrenalectomized rats.

DISCUSSION

Previous investigations of the effects of α and β blockers in liver metabolism[19] showed sensitivity to both classes of compounds; in rat liver, glycogenolysis induced by noradrenaline was prevented by 5 mg/kg phenoxybenzamine.[20] Attempts to define the site of action of this drug by autoradiography have so far proved unsuccessful;[21] the results here show that 5 mg/kg phenoxybenzamine administered simultaneously with ^{3}H thymidine did not affect ^{3}H incorporation into DNA, indicating that at this dose the blocker does not directly affect the enzymes involved in DNA synthesis.

The action of phenoxybenzamine given immediately before partial hepatectomy suggests that catecholamines acting through α sites promote enzyme induction early in liver regeneration. The effects of later administration after ornithine decarboxylase induction indicate that metabolic changes accompanying the appearance of this enzyme are not themselves normally sufficient to promote DNA replication, and that catecholamines still facilitate the second wave of enzyme induction between 12–15 hr after operation. Whether this is entirely consequential to the activation of plasma membrane adenyl cyclase still requires elucidation. The influence of phenoxybenzamine on nuclear histone phosphokinase levels is reminiscent of the delayed appearance of this enzyme following radiation given early in G_1[14] but the failure of irradiation to prevent induction of ornithine decarboxylase[22] underlines the complexity of the processes contributing to enzyme induction *in vivo*. The lack of effect of the α blocker on the increased entry of orotic acid into liver 1 hr after operation also shows that membrane changes associated with enzyme induction may be distinguished from those influencing its permeability properties.

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