Biochemical Pharmacology, Vol. 28, pp. 3539–3544.
© Pergamon Press Ltd. 1979. Printed in Great Britain.
0006-2952/79/1215-3539 $02.00/0

EFFECTS OF ADRENERGIC ANTAGONISTS ON PREREPLICATIVE CHANGES DURING NAFENOPLIN-INDUCED LIVER GROWTH IN THE RAT*

DAVID C. C. LAM and WALTER G. LEVINE

Department of Molecular Pharmacology, Albert Einstein College of Medicine Yeshiva University, Bronx, NY 10461, U.S.A.

(*Received* 21 *February* 1979; *accepted* 4 *June* 1979)

Abstract—Nafenopin (NP) induces hepatomegaly characterized by cellular hypertrophy and hyperplasia. An investigation was carried out on the effects of adrenergic antagonists on prereplicative changes in the livers of rats treated with NP. After a single dose of 200 mg/kg, induction of amino acid uptake (AAU) was maximal at 9 hr, induction of ornithine decarboxylase (ODC) activity was biphasic with peaks at 9 and 21 hr, and DNA synthesis was maximally induced at 21 hr. The α-adrenergic antagonists phenoxybenzamine and phentolamine, injected 0.5 hr before or 3 hr after NP, further enhanced AAU and ODC activity at 9 hr. The β-adrenergic antagonists propranolol and pindolol elicited a similar enhancement (superinduction) when injected 0.5 hr before but not 3 hr after NP. When α- and β-antagonists were injected simultaneously, no net enhancement of NP-induced AAU or ODC was seen at 9 hr. Enhancement of the 21 hr peak of ODC activity is seen in response to α- and β-antagonists injected at 12 hr but not at 3 hr. Despite the marked stimulation in AAU and ODC activity in response to the antagonists, DNA synthesis was not affected. Inferences drawn from these results are: (1) α- and β-receptors may interact to exert a modulating affect on NP induction such that blockade of one site elicits superinduction while blockade of both sites does not significantly alter the NP response, (2) there appear to be time-related changes in these receptor relationships after administration of NP, (3) the superinduction of AAU and ODC associated with α-antagonists injected 3 hr after NP may specifically involve β-receptors, (4) the two peaks of ODC activity are at least partially independent of each other, and (5) the adrenergic receptor mechanisms responding to administration of NP are somewhat different from those functioning during liver regeneration.

Nafenopin (NP, SU-13437), a hypolipidemic compound, causes massive liver enlargement accompanied by hepatocellular hyperplasia and proliferation of microbodies and smooth endoplasmic reticulum in mice and rats [1–3] with little or no effect on hepatic microsomal drug-metabolizing enzymes [4, 5]. After intraperitoneal administration of NP, increases in ornithine decarboxylase (ODC) activity and amino acid uptake (AAU) have been observed at 9 hr while an increase in DNA synthesis has been observed at 21 hr [6]. Similar responses have also been reported in the regenerating liver after partial hepatectomy [7–9]. Morphologically, a NP-induced liver resembles regenerating liver [10].

Adrenergic receptor mechanisms probably play a role in regenerating liver. Increases in cyclic AMP levels have been seen at 4 and 12 hr after partial hepatectomy [11–13]. Adrenergic receptor activation has been associated with changes in liver cyclic AMP levels [14] and adrenergic blocking agents have been used to establish this linkage. The alpha-adrenergic blocking agents phenoxybenzamine and phentolamine, injected at the time of partial hepatectomy, inhibited the induction of ODC seen normally at 4 hr [13]. Given at 8 hr, these agents inhibited the second wave of cyclic AMP accumulation and subsequent DNA synthesis [12]. Administration of the beta-adrenergic blocking agents propranolol and pindolol, 1 hr after partial hepatectomy decreased the first wave of cyclic AMP with no effect on ODC activity or on DNA synthesis [13]. However, when *dl*-propranolol was administered at 8 hr, the second wave of cyclic AMP and subsequent DNA synthesis were delayed [12].

It has been proposed [15] that induction by 3-methylcholanthrene and phenobarbital of ODC and of mixed-function oxidase activity, as well as subsequent liver hypertrophy, is mediated by increases in cyclic AMP through beta-adrenergic receptors. In livers of rats fed 3′-methyl-4-dimethylaminoazo benzene [16] and in hepatocytes isolated from rats fed with 2-acetylamino fluorene [17], there is a propranolol-sensitive increase in the response of adenyl cyclase activity to isoproterenol. Since adrenergic receptor blockade leads to change in live cyclic AMP, ODC activity, and DNA synthesis in partially hepatectomized rats and possibly in chemically induced liver growth, we investigated the influence of α- and β-adrenergic blocking agents on several prereplicative changes seen during NP-induced liver growth.

MATERIALS AND METHODS

Chemicals. Pyridoxal phosphate, dithiothreitol, diphenylamine and *dl*-propranolol were purchased from the Sigma Chemical Co. (St. Louis, MO), EDTA from Fluka-Garantie (Switzerland) and Hyamine hydroxide (10-X) from the Packard Instru-

* This work was supported in part by a grant from the National Cancer Institute, USPHS (CA-14231).

ment Co. (Downers Grove, IL). Aquasol and *dl*-[1-^{14}C]ornithine were obtained from New England Nuclear (Boston, MA). 2-Amino[1-^{14}C]isobutyric acid (59 mCi/mmole) and [*methyl*-^{3}H]thymidine (19 Ci/mmole) were purchased from Amersham (Arlington Heights, IL) and Schwarz/Mann (Orangeburg, NY) respectively. Nafenopin and phentolamine (Regitine HCl) were provided by the Ciba Pharmaceutical Co. (Summit, NJ), phenoxybenzamine (Dibenzyline) by Smith, Kline & French Laboratories (Philadelphia, PA), and pindolol (LB-46) by Sandoz Pharmaceuticals (East Hanover, NJ).

Treatment of animals. Male Wistar rats (initial body weights, 40–50 g) were kept under controlled lighting (8:00 p.m.–8:00 a.m.) and feeding (8:00 a.m.–1:00 p.m.) conditions for 2–3 weeks.

Water was available at all times. NP was dissolved in corn oil (75 mg/ml) and injected intraperitoneally at a dose of 200 mg/kg. Control animals received an equal volume of vehicle.

Amino acid uptake (AAU) and ornithine decarboxylase (ODC) activity. AAU and ODC activity were determined on the same liver 9 hr after injection of NP. 2-Amino[1-^{14}C]isobutyric acid, a non-metabolized amino acid [18], was injected intravenously, 2 μCi/100 g. The livers were removed 5 min later, perfused with ice-cold saline, and homogenized in 4 vol. of ice-cold 50 mM phosphate buffer, pH 7.4, containing 1 mM dithiothreitol, 0.1 mM EDTA and 0.1 mM pyridoxal phosphate (added fresh daily). For determination of AAU, 2 ml of the homogenate were mixed with 2 ml of 10% trichloroacetic acid (TCA), and the acid-soluble radioactivity was collected after centrifugation at 100 *g*. The pellet was washed once with an equal volume of 5% TCA, the supernatant fractions were combined, and a sample was counted. Uptake was calculated as cpm/g of liver. For determination of ODC activity, the remaining homogenate was centrifuged for 60 min at 100,000 *g* and the supernatant fractions were frozen overnight. It was found that overnight storage in the freezer had no effect on the enzyme activity although activity diminished after 2–3 days of storage (data not shown). For the assay, 1.8 ml of the buffered supernatant fraction, to which had been added freshly prepared pyridoxal phosphate, were preincubated for 10 min at 37°. The reaction was started with the addition of 0.2 ml *dl*-[1-^{14}C]ornithine (0.5 μCi). The final concentration of ornithine was 0.6 mM. The flasks were capped and incubated at 37° for 30 min in a water bath with constant shaking. The reaction was stopped by the addition of 0.2 ml of 5 N H_2SO_4. The flasks were incubated for another 30 min and the $^{14}CO_2$ evolved was trapped by 0.2 ml Hyamine hydroxide in methanol. The cup containing the Hyamine was mixed with 5 ml Aquasol and cooled overnight before determining the total radioactivity. Enzyme activity was calculated as pmoles CO_2 evolved/mg of protein/30 min.

DNA synthesis. DNA synthesis and ODC activity were also determined on the same liver 21 hr after injection of NP. Rats were injected intraperitoneally with 30–35 μCi [^{3}H]thymidine. One hr later the livers were removed and homogenized in 4 vol. of ice-cold 50 mM phosphate buffer, pH 7.4, containing 1 mM dithiothreitol, 0.1 mM EDTA, and 0.1 mM pyridoxal phoshate (prepared fresh). A sample of the homogenate was mixed with an equal volume of ice-cold 10% TCA and centrifuged. The precipitate was washed twice with 5% TCA, twice with ethanol, and twice with hot ethanol–ethyl ether (3:1; v/v). Finally, the DNA was extracted in hot 5% TCA and a sample was counted. DNA was determined by the diphenylamine procedure [19], using calf thymus DNA as a standard.

Neither the presence of 2-amino[1-^{14}C]isobutyric acid nor that of [^{3}H]thymidine interferes with the ODC assay (data not shown). Protein was determined by the procedure of Lowry *et al.* [20] with crystalline bovine albumin as a standard. Statistical analysis was determined by Student's *t*-test method.

RESULTS

Enhanced liver uptake of 2-amino-isobutyric acid and ODC activity are seen 9 hr after i.p. administration of NP [6]. Determination was made on the effects on these parameters of adrenergic blocking agents administered at various times before or after NP. In Table 1, it is seen that the α-adrenergic antagonists phenoxybenzamine and phentolamine, injected 0.5 hr before or 3 hr after NP treatment, enhanced the NP-induced AAU by approximately 150–200 per cent. β-Adrenergic antagonists, pindolol or propranolol, elicited similar enhancement when injected 0.5 hr before but not 3 hr after NP. In certain instances, a single antagonist alone appeared to increase AAU, in the absence of NP, possibly due to a stress effect. However, none of these increases were statistically significant and the enhancement of NP-induced AAU was not simply an additive effect. When α-and β-antagonists were injected together in the absence of NP, a small but significant increase was seen. However, when this increase was taken into account, the combined antagonists given 0.5 hr before or 3 hr after NP caused no significant net enhancement of AAU above levels seen with NP alone.

We extended the earlier observations of Levine *et al.* [6] and found a second peak of ODC activity

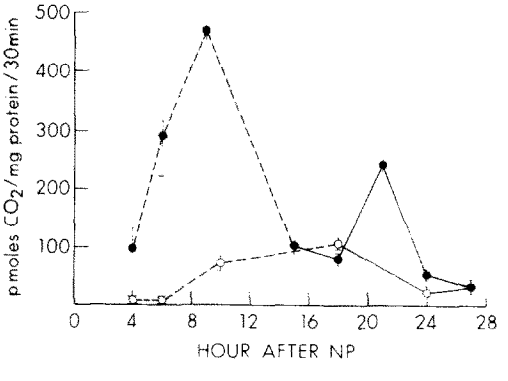

Fig. 1. Time course of ODC activity after NP administration. Each point represents the mean ± S.E.M. for three animals. Key: (○) control; (●) NP-treated (---) data from Levine *et al.* [6]

Table 1. Effects of α- and β-adrenergic antagonists on amino acid uptake at 9 hr*

Treatment	Time of administration (hr)	Amino acid uptake (cpm/g liver)
Control (corn oil)	0	31,089 ± 2319
Phenoxybendamine	−0.5	44,177 ± 2710
NP	0	70,792 ± 1202†
+ Phenoxybendamine	−0.5	210,187 ± 37,156‡
Control (corn oil)	0	35,325 ± 698
Pindolol	−0.5	23,789 ± 2,013
NP	0	56,973 ± 6,903†
+ Pindolol	−0.5	116,116 ± 10,177‡
Control (corn oil)	0	25,192 ± 918
NP	0	52,681 ± 4,724†
+ Propranolol	−0.5	151,191 ± 24,099‡
Control (corn oil)	0	12,442 ± 642
Propranolol	−0.5	16,700 ± 2,383§
Control (corn oil)	0	21,488 ± 5,888
Phenoxybendamine + pindolol	−0.5	52,296 ± 17,656
NP	0	66,045 ± 10,336†
+ Phenoxybenzamine + pindolol	−0.5	124,397 ± 25,598
Control (corn oil)	0	21,589 ± 1,175
Phenoxybenzamine	3	20,827 ± 3,677
Phentolamine	3	29,697 ± 3,480
Propranolol	3	40,899 ± 4,044†
NP	0	65,524 ± 10,122†
+ Phenoxybenzamine	3	158,798 ± 22,905‡
+ Phentolamine	3	151,891 ± 24,664‡
+ Propranolol	3	80,133 ± 11,953
Control (corn oil)	0	16,699 ± 3,919
Phenoxybenzamine + propranolol	3	46,364 ± 9,324†
NP	0	59,873 ± 15,573†
+ Phenoxybenzamine + propranolol	3	130,330 ± 5,812‡
Phentolamine + pindolol	3	46,926 ± 8,721†
NP	0	69,978 ± 2,682†
+ Phentolamine + pindolol	3	125,647 ± 22,910‡

* Male rats (100–150 g) were injected with NP (200 mg/kg, i.p.). Phenoxybenzamine (10 mg/kg), phentolamine (10 mg/kg), propranolol (60 mg/kg), and pindolol (5 mg/kg) were injected i.p. as indicated. [^{14}C]-2-Amino-isobutyric acid was injected intravenously (2 μCi/1000 g). Five min later the TCA-soluble radioactivity was determined in the prefused livers. Each value represents the mean ± S.E.M. for three or four animals.

† Versus control ($P < 0.05$).

‡ Versus NP ($P < 0.05$).

§ Control group was determined separately.

at 21 hr (Fig. 1). This peak coincided with the peak of DNA synthesis after NP administration [6]. Several investigators have also reported multiple peaks of ODC during liver regeneration [21–24]. Figure 2 shows the effects of the adrenergic antagonists on ODC activity measured at 9 hr and 21 hr after NP. The alpha-blockers, phenoxybenzamine and phentolamine, injected 0.5 hr before (Fig. 2A) or 3 hr after (Fig. 2B), led to a superinduction of liver ODC activity at 9 hr. Superinduction was also seen with the beta-blockers, propranolol or pindolol, injected 0.5 hr before but not 3 hr after NP. Neither antagonist injected alone produced a significant increase in ODC activity. When α- and β-antagonists were injected together at either time, no statistically significant enhancement of ODC activity at 9 hr was seen (Table 2). Injection of either α- or β-antagonists 12 hr after NP also markedly enhanced the second peak of ODC activity at 21 hr (Fig. 2C). When the antagonists were injected 0.5 hr before or 3 hr after NP, no alteration of the second ODC peak was seen, despite induction of the first peak at 9 hr (Fig. 2 panels A and B). Thus, there appeared to be a degree of independence between the two ODC peaks. In contrast to the 9 hr ODC peak, the 21 hr peak was further stimulated by combined injection of α- and β-antagonists (Table 2).

DNA synthesis was stimulated markedly after NP treatment (Fig. 3). However, neither phenoxybenzamine, nor phentolamine, nor propranolol, nor pindolol, injected at any time, produced significant effects on NP-induced [^{3}H]thymidine incorporation into DNA. These results are in contrast to those reported in regenerating liver by Thrower and Ord [13] and by MacManus *et al.* [12], who demonstrated that phenoxybenzamine, as well as propranolol, injected 8–13 hr after partial hepatectomy, delayed the induction of DNA synthesis.

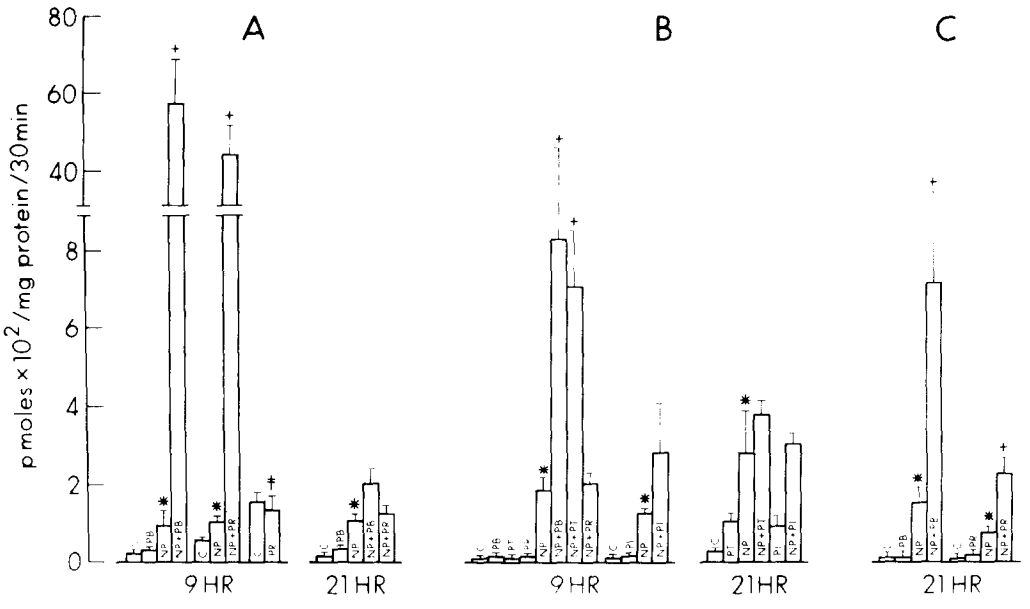

Fig. 2. Changes in ODC activity in response to NP and adrenergic blocking agents. Doses used: NP (200 mg/kg), phenoxybenzamine (PB; 10 mg/kg), phenotolamine (PT; 10 mg/kg), propranolol (PR; 60 mg/kg), and pindolol (PI; 5 mg/kg). Antagonists were injected i.p. 0.5 hr before (A), 3 hr after (B), or 12 hr after (C) NP. Enzyme activity was measured 9 or 21 hr after NP, as indicated. Each value represents the mean ± S.E.M. for three or four rats Key: (*) versus control ($P < 0.05$); (†) versus NP ($P < 0.05$); (‡) control group determined separately.

DISCUSSION

The superinduction response to adrenergic blocking agents in the presence of NP suggests that the induction of AAU and of ODC by NP may involve adrenergic receptor mechanisms. It is doubtful that the enhanced response to the individual agents is simply a stress effect since the drugs in the absence of NP elicit minimal changes which are far below those seen in the presence of NP, especially in the case of ODC. Furthermore, combined α- and β-blockers given 0.5 hr before NP abolish the superinduction response. β-Adrenergic receptor mechanisms have also been postulated to play a role in the

Table 2. Effects of α- and β-antagonists together with ODC activity at 9 and 21 hr*

Treatment	Time of administration (hr)	Time (hr)	ODC activity pmoles/mg protein/30 min
Control (corn oil)	0	9	25 ± 3
Phenoxybenzamine + pindolol	−0.5	9	37 ± 13
NP	0	9	509 ± 138†
+ Phenoxybenzamine + pindolol	−0.5	9	1436 ± 555‡
Control (corn oil)	0	9	18 ± 4
Phenoxybenzamine + propranolol	3	9	1088 ± 133†
NP	0	9	336 ± 67†
+ Phenoxybenzamine + propranolol	3	9	1284 ± 836‡
Control (corn oil)	0	9	56 ± 4
NP	0	9	254 ± 137§
+ Phentolamine + pindolol	3	9	862 ± 307‡
Control (corn oil)	0	21	42 ± 4
Phenoxybenzamine + pindolol	12	21	33 ± 10
NP	0	21	134 ± 42
+ Phenoxybenzamine + pindolol	12	21	755 ± 1808

* Male rats (100–150 g) were injected with NP (200 mg/kg, i.p.). Phenoxybenzamine (10 mg/kg), phentolamine (10 mg/kg), propranolol (60 mg/kg), and pindolol (5 mg/kg) were injected i.p. at the various times indicated. Ornithine decarboxylase activity was determined as described in Materials and Methods. Each value represents the mean ± S.E.M. for three or four animals.

† Versus control ($P < 0.05$).

‡ Versus NP (not significant).

§ Versus NP ($P < 0.03$).

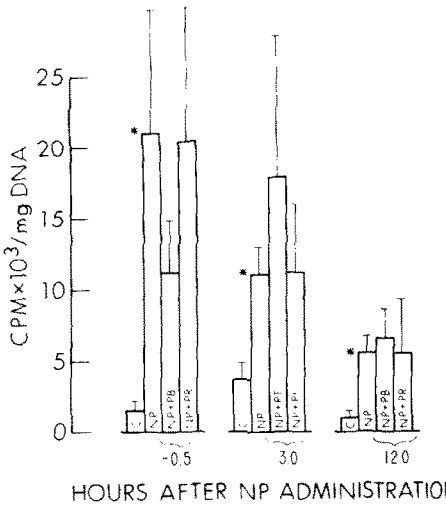

Fig. 3. Effects of adrenergic antagonists on NP-induced DNA synthesis. NP (200 mg/kg) was injected i.p. at zero time; phenoxybenzamine (PB; 10 mg/kg), phentolamine (PT; 10 mg/kg), propranolol (PR; 60 mg/kg, and pindolol (PI; 5 mg/kg) were injected i.p. as indicated. Refer to Materials and Methods for details. Each value represents the mean ± S.E.M. of three or four rats. Key: (*) versus control ($P < 0.05$).

induction of ODC in the regenerating liver [25] and of the microsomal mixed-function oxygenase system and liver hypertrophy by phenobarbital, 3-methylcholanthrene and Aroclor-1254 [15, 26, 27].

It is apparent from present and earlier observations [6] that NP induces two peaks of ODC activity. The first peak is at 9 hr and the second is at 21 hr and coincides with maximal DNA synthesis. Both α- and β-blocking agents injected 0.5 hr before NP lead to a superinduction of NP-stimulated AAU (Table 1) and ODC activity (Fig. 2, panels A and B) at 9 hr. However, when injected 3 hr after NP, only the α-antagonists effectively enhance AAU and ODC activity (Fig. 2, panels A and B). Injection of both antagonists together 0.5 hr before or 3 hr after NP produces no superinduction of either activity (Tables 1 and 2). There is also superinduction of the second ODC peak by α- or β-antagonists injected 12 hr after NP. In contrast to the effects on the earlier peak, combined injection of α- and β-antagonists still elicits a superinduction of the 21 hr peak.

The mechanism of the superinduction is thus far not clearly defined, although both induction by NP alone and superinduction appear to require protein synthesis since they are inhibited by cycloheximide [28]. One possibility is that adrenergic receptors control repressor mechanisms in the induction process. Thus, receptor blockade would effectively "derepress" the NP response leading to superinduction. The obliteration of superinduction by the simultaneous administration of both α- and β-blockers argues against this explanation since, under these conditions, an even greater degree of superinduction would have been expected. Alternatively, the α- and β-receptor sites may serve as a precisely balanced modulating influence on NP induction such that blockade of either site alone may lead to an exaggerated response (superinduction), while blockade of both sites removes the influence entirely. The concept of a linked α- and β-receptor modulating effect is also implicit in the work of McCarthy and deVellis [29], who studied the norepinephrine-induced increase in levels of cAMP in glial cultures of rat cerebral cortex. Alpha-adrenergic blockade with phentolamine led to a marked superinduction of this response, while combined β-blockade with propranolol abolished the increase. On the other hand, Le Cam and Freychet [30] observed induction of AAU by catecholamines in isolated rat hepatocytes which was blocked by α- but not β-antagonists. Although these results are ostensibly the opposite of ours where adrenergic antagonists enhanced AAU, they do indicate an involvement of adrenergic receptor mechanisms in AAU.

We have found a time-related change in the response to adrenergic blocking agents. Thus, both α- and β-blockade individually elicit superinduction when given 0.5 hr prior to NP, while 3 hr after NP superinduction is seen only after α-blockade. By 12 hr, the response to β-blockade has returned, but now simultaneous administration of both α- and β-blockers still elicits superinduction, possibly implying an alternative mechanism of induction. A change in the relationship between α- and β-receptor responsiveness has been reported in hepatocytes obtained from the regenerating liver [31]. With the use of adrenergic antagonists, it was demonstrated that the agonist-induction of glycogen phosphorylase is mediated via α-receptors in the normal adult liver, while 24 hr after partial hepatectomy the effect is mediated via β-receptors. Thus, both NP administration and partial hepatectomy may induce a rapid change in adrenergic receptor relationships.

The use of these antagonists has allowed us to examine the relationship between the two ODC peaks at 9 and 21 hr. Injection of adrenergic antagonists 0.5 hr before or 3 hr after NP results in a marked stimulation of ODC activity at 9 hr, but not at 21 hr (Fig. 2A). The second peak is, however capable of superinduction, as seen after injection of antagonists 12 hr after NP. Thus, it is possible to influence one peak of ODC activity but not the other. Independence of the two ODC activity peaks was also reported in regenerating livers by McGowan and Fausto [24]. They demonstrated that protein deprivation did not affect the first increase in ODC activity while the second peak was delayed. Similarly, they showed that hypophysectomy markedly inhibited the first ODC activity peak in regenerating livers without affecting the second peak when compared to nonhypophysectomized rats.

Finally, our results with adrenergic antagonists on NP-induced ODC activity and DNA synthesis contrast with those reported in the regenerating liver. Thrower and Ord [13] found that α-antagonists, given at the time of partial hepatectomy, delay ODC induction without affecting DNA synthesis. However, when either class of antagonist was given 9–12 hr after partial hepatectomy, DNA synthesis was delayed [12, 13]. Ashrif *et al.* [32] showed than DNA synthesis during regeneration was not affected by α-blockade (tolazoline) but was inhibited by β-blockade (propranolol) and the sympathetic nerve block-

ers, guanethide and reserpine. In the present work we show that administration of α- as well as β-antagonists can lead to superinduction of both peaks of NP-stimulated ODC activity (Fig. 2, panels A and B), but under no circumstances is DNA synthesis affected (Fig. 3). It is apparent that certain aspects of the prereplicative events responding to NP differ from those seen in the regenerating liver.

REFERENCES

1. R. Hess, R. Maiser and W. Staubli, *Adv. exp. Med. Biol.* **4**, 483 (1969).
2. J. Reddy, D. Svoboda and D. Azarnoff, *Biochem. biophys. Res. Commun.* **52**, 537 (1973).
3. W. G. Levine, *Drug Metab. Dispos.* **2**, 178 (1974).
4. R. B. Beckett, R. Weiss, R. E. Stitzel and R. J. Cendalla, *Toxic. appl. Pharmac.* **23**, 42 (1972).
5. W. G. Levine and J. Bognacki, *J. Pharmac. exp. Ther.* **196**, 486 (1976).
6. W. G. Levine, M. G. Ord and L. A. Stocken, *Biochem. Pharmac.* **26**, 939 (1977).
7. D. Russell and S. H. Synder, *Proc. natn. Acad. Sci. U.S.A.* **60**, 1420 (1968).
8. N. L. R. Bucher and N. J. Oakman, *Biochim. biophys. Acta* **186**, 13 (1969).
9. J. Short, N. B. Armstrong, R. Zemel and I. Lieberman, *Biochem. biophys. Res. Commun.* **50**, 430 (1973).
10. A. B. Novikoff, P. M. Novikoff, M. Mori and W. G. Levine, *J. Histochem. Cytochem.* **23**, 314 (1975).
11. J. P. MacManus, D. J. Franks, T. Youdale and R. M. Braceland, *Biochem. biophys. Res. Commun.* **49**, 1201 (1972).
12. J. P. MacManus, B. M. Braceland, T. Youdale and J. F. Whitfield, *J. cell. Physiol.* **82**, 157 (1973).
13. S. Thrower and M. G. Ord, *Biochem. J.* **144**, 361 (1974).
14. G. A. Robison, R. W. Butcher and E. W. Sutherland, in *Cyclic-AMP*, p. 153. Academic Press, New York (1971).
15. C.-A. Manen, M. Costa, I. G. Sipes and D. H. Russell, *Biochem. Pharmac.* **27**, 219 (1978).
16. H. Boyd and T. J. Martin, *Molec. Pharmac.* **13**, 576 (1977).
17. H. Boyd and T. J. Martin, *Cancer Res.* **34**, 1720 (1974).
18. H. N. Christensen, A. J. Aspen and E. G. Rice, *J. biol. Chem.* **220**, 287 (1956).
19. H. N. Munro and A. Fleck, in *Methods of Biochemical Analysis* (Ed. D. Gill), Vol. 14, p. 113. Interscience, London (1966).
20. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
21. E. Holtta and J. Janne, *Fedn. Eur. Biochem. Soc. Lett.* **23**, 117 (1972).
22. D. J. Gaza, J. Short and I. Lieberman, *Fedn. Eur. Biochem. Soc. Lett.* **32**, 251 (1973).
23. B. Barbiroli, M. S. Moruzzi, B. Tadolini and M. G. Mouti, *J. Nutr.* **105**, 408 (1975).
24. J. A. McGowan and N. Fausto, *Biochem. J.* **170**, 123 (1978).
25. C. V. Byus, G. A. Hedge and D. H. Russell, *Biochim. biophys. Acta* **498**, 39 (1977).
26. M. Costa, E. R. Costa, C.-A. Manen, G. Sipes and D. H. Russell, *Molec. Pharmac.* **12**, 871 (1976).
27. C. V. Byus, M. Costa, I. G. Sipes, B. B. Brodie and D. H. Russell, *Proc. natn. Acad. Sci. U.S.A.* **73**, 1241 (1976).
28. D. C. C. Lam and W. G. Levine, *Biochem. biophys. Res. Commun.*, in press.
29. K. D. McCarthy and J. deVellis, *J. Cyclic Nucleo. Res.* **4**, 15 (1978).
30. A. Le Cam and P. Freychet, *Endocrinology* **102**, 379 (1978).
31. K. R. Hornbrook, *Pharmacologist* **20**, 166 (1978).
32. S. Ashrif, J. S. Gillespie and D. Pollock, *Eur. J. Pharmac.* **29**, 324 (1974).