MOLECULAR PHARMACOLOGY, 13, 576-578

SHORT COMMUNICATIONS

The Effect of Propranolol on Induction of Rat Liver Tumors by a Chemical Carcinogen

HELEN BOYD[1] AND T. J. MARTIN[2]

Department of Medicine, University of Melbourne, Austin Hospital, Heidelberg, Victoria 3084, Australia

(Received October 1, 1976)
(Accepted November 30, 1976)

SUMMARY

BOYD, HELEN & MARTIN, T. J. (1977) The effect of propranolol on induction of rat liver tumors by a chemical carcinogen. *Mol. Pharmacol.*, 13, 576-578.

Previous work has indicated that during chemical carcinogenesis in rat liver there is an increase in the catecholamine responsiveness of adenylate cyclase. To investigate what role the increased *beta* adrenergic responsiveness of the enzyme might play in neoplastic transformation, the effect of the *beta* adrenergic blocker propranolol on tumor incidence in rat liver has been investigated during treatment with the carcinogen 3'-methyl-4-dimethylaminoazobenzene. Propranolol, administered in the drinking water during carcinogen treatment, approximately doubled tumor incidence. It is concluded that increased *beta* adrenergic responsiveness is unlikely to be a process causal to cancer and, moreover, that propranolol somehow facilitates neoplastic transformation in this system. Whether such an effect of propranolol is due to specific *beta* blockade or to nonspecific actions remains to be elucidated.

INTRODUCTION

Increases in catecholamine responsiveness of adenylate cyclase have been observed by us (1, 2), and by others (3-6), in the livers of rats undergoing neoplastic transformation in response to dietary intake of the chemical carcinogens 3'-methyl-4-dimethylaminoazobenzene and 2-acetylaminofluorene. These changes were first observed in washed particle preparations from whole liver containing a mixed population of cells (3, 1), and then in tissue slices (4) and preparations of isolated parenchymal cells (5). They were shown to be accompanied by changes in intracellular levels of cyclic 3', 5'-AMP in whole liver slices (6).

Adenylate cyclase activity in the washed particles from preneoplastic liver studied by us not only was increased in its responsiveness to the stimulatory (*beta* adrenergic) effects of catecholamines, but had an elevated basal level, against which an increased responsiveness to the inhibitory (*alpha* adrenergic?) effects of adrenergic agents were seen. *l*-Propranolol [1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol] blocked stimulation and unmasked inhibition of the enzyme by catecholamines (2).

Since it was possible that the changes in adenylate cyclase observed were important to neoplastic transformation, we decided to test the effect of propranolol on the rate of onset of tumors in rats fed 3'-methyl-4-dimethylaminoazobenzene. A diet of chow containing 0.06% 3'-methyl-4-

This work was supported by the Australian Tobacco Foundation and the National Health and Medical Research Council of Australia.

[1] Present address, Department of Pharmacology, University of Miami Medical School, Miami, Florida 33152.

[2] Present address, Department of Chemical Pathology, University of Sheffield, Sheffield S10 2RX, England.

ISSN 0026-895X

TABLE 1

Effect of propranolol on incidence of tumors in rat livers

Treatment	Propranolol	Fraction of total animals with tumors			Fraction of total liver lobes with tumors		
		Day 96	Day 107	Day 118	Day 96	Day 107	Day 118
	%						
Control		0/7	0/8	0/8	0/14		0/16
+Propranolol	0.05	0/7		0/8	0/14		0/16
	0.10		0/8			0/16	
Carcinogen							
+Propranolol		4/8	4/8	5/8	4/16		8/20
	0.02						
	0.05	7/8	6/8	9/10	9/16		11/14
	0.10						
	0.50	7/7	8/8	8/8	10/14		16/16

dimethylaminoazobenzene caused tumors in 90–100% of the animals by 20 weeks and in about 50% by 12–13 weeks. We reasoned that around 12–13 weeks would be an appropriate time to look for any change in tumor incidence.

It had been established in animal trials that propranolol could be administered to rats in their drinking water, and that a dosage of 0.2 mg/kg administered subcutaneously twice daily lowered the blood pressure of hypertensive rats to normal, without causing bradycardia. However, it appeared that 75 mg/kg orally would be necessary for therapeutic effects on some cardiovascular abnormalities.[3] Because of the relative resistance of liver adrenergic receptors *in vivo* to *beta* adrenergic blockade (7, 8), it seemed wise to treat the rats with high doses of propranolol in order to be sure of preventing the *beta* adrenergic stimulation of liver adenylate cyclase which would occur with the endogenous release of catecholamines during the normal activities of the rats. Since rats (male, more than 200 g) drink approximately 15 ml of water per day, *dl*-propranolol in the range of 0.02–0.5% was administered in the drinking water. The rats (male, Sprague-Dawley, more than 200 g at the beginning of treatment) were thus given dosages of approximately 15–375 mg/kg/day. [Therapeutic human oral dosages are much lower: viz. 1–60 mg/kg/day, generally 1–5 mg/kg/day (9).]

[3] C. Proctor, personal communication.

Rats were housed in cages containing groups of four or five to minimize stress from under- or overcrowding, and kept in rooms with controlled lighting which also received natural daylight. Propranolol solutions were made fresh each day. Control and carcinogen-containing diets were supplied as previously described (1, 2). Rats were killed on days 96, 107, and 118, and their livers were examined visually and histologically (1) for tumors. Tumor incidence is shown in Table 1. One could further quantitate the tumor incidence by ascertaining the number of lobes of each liver which contained tumors, and also by assessing the size of the tumors. The latter was not done, but the observation was that in the propranolol-treated animals the tumors were larger. The former was done on days 6 and 118; Table 1 expresses these results as a fraction of the total number of liver lobes.

It is clear that tumor incidence increased dramatically with propranolol, in a manner dependent upon the concentration of the drug. It should be emphasized that the dosages used were considerably higher than those generally used clinically in humans. It is not clear whether this effect of propranolol was due to specific *beta* adrenergic blockade or to nonspecific actions. Nor is it clear whether this effect is due to direct action on the liver cells or, for example, to an effect on appetite (to alter intake of carcinogen) or on the immune system. Further studies are required to resolve these questions, and also

the question of the dose range in which the co-carcinogenic property of this widely used therapeutic agent is seen. Such studies are in progress with the cooperation of Ayerst Laboratories, which markets propranolol in the United States. Another *beta* adrenergic blocking agent, pronethalol, was withdrawn from the market because it caused tumors in mice, although not in rats (10).

We conclude that the increased responsiveness of adenylate cyclase to stimulation by catecholamines during carcinogenesis was not causal to neoplasia, since its blockade did not prevent tumor formation. However, it is interesting that low doses of catecholamines inhibited adenylate cyclase activity in preneoplastic liver to a greater extent than it was possible to observe in normal liver, and that propranolol enhanced this effect (2). The possibility of a role of these apparent *alpha* adrenergic effects of catecholamines in neoplastic transformation remains to be elucidated.

ACKNOWLEDGMENTS

We wish to express our gratitude to Karl Liebel for his help with these experiments, and to ICI for a gift of propranolol.

REFERENCES

1. Boyd, H., Martin, T. J. & Louis, C. J. (1974) *Cancer Res.*, 34, 1720–1725.
2. Boyd, H. & Martin, T. J. (1976) *Mol. Pharmacol.*, 12, 195–202.
3. Christoffersen, T., Mørland, J., Osnes, J. B. & Elgjo, K. (1972) *Biochim. Biophys. Acta*, 279, 363–366.
4. Christoffersen, T., Brønstad, G. O., Walstad, P. L. & Øye, I. (1974) *Biochim. Biophys. Acta*, 372, 291–303.
5. Christoffersen, T. & Berg, T. (1975) *Biochim. Biophys. Acta*, 381, 72–77.
6. Christoffersen, T. (1975) *Acta Pharmacol. Toxicol.*, 37, 233–236.
7. Kennedy, B. L. & Ellis, S. (1969) *Arch. Int. Pharmacodyn. Ther.*, 177, 390–406.
8. Newton, N. E. & Hornbrook, K. R. (1972) *J. Pharmacol. Exp. Ther.*, 18, 479–488.
9. Shand, D. G. (1974) *Dis. Month*, Oct., 1–30.
10. Paget, G. E. (1963) *Br. Med. J.*, 2, 1266–1267.