RAPID COMMUNICATION Jpn. J. Cancer Res. 81, 557–559, June/July 1990

Changes in Number of α-Adrenergic Receptor Subtypes in Hepatocytes from Rats Fed 3′-Methyl-4-dimethylaminoazobenzene

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Changes in numbers of α_1- and α_2-adrenergic receptors in the plasma membranes of hepatocytes from female Donryu rats given feed containing 0.06% of the carcinogen 3′-methyl-4-dimethylaminoazobenzene (3′-MeDAB), were examined. α_1-Adrenergic receptors, measured in terms of [^{3}H]-prazosin binding, decreased to half of the control 2 weeks after the start of this diet, then gradually decreased for the next 22 weeks. α_2-Adrenergic receptors, measured in terms of [^{3}H]clonidine binding, transiently increased 3-fold over the control at 2 weeks. These changes in the early period of the 3′-MeDAB diet intake may be related to hepatocarcinogenesis.

Key words: 3′-Methyl-4-dimethylaminoazobenzene — Hepatocyte — α_1-Adrenergic receptor — α_2-Adrenergic receptor

Hormones regulate many cell functions in various tissues. In tumor cells, uncontrolled growth may result from some malignant transformation involving changes in the hormone receptors.[1,2]

Treatment of rats with chemical carcinogens, including 2-acetylaminofluorene (2-AAF) and 3′-methyl-4-dimethylaminoazobenzene (3′-MeDAB), increases the hepatic catecholamine-sensitive adenylate cyclase activation and the ability of the liver tissue to form cyclic AMP in response to adrenergic activation.[1-6] Refsnes *et al.*[2] have indicated that an increase in the number of β-adrenergic receptors occurs in hepatocytes from rats treated with 2-AAF and suggested that this might explain the rise in catecholamine-sensitive adenylate cyclase activity. However, there are few reports on changes of α-adrenergic receptors during the induction of hepatocarcinogenesis.

We have shown that rat ascites hepatoma (AH) 130 cells, which were induced by DAB and established as a transplantable tumor, have many β_1- and α_2-adrenergic receptors,[7-9] while hepatocytes from normal adult rats have β_2- and α_1-adrenergic receptors. We are interested in the switchover of the receptor subtypes during carcinogenesis. In this report, we describe changes in the numbers of α-adrenergic receptor subtypes in rat hepatocytes during *in vivo* 3′-MeDAB administration.

Female Donryu rats (Shizuoka Laboratory Animal Center, Hamamatsu), weighing 100–150 g at the beginning of the experiment, were used. The animals were fed *ad libitum* a diet (CE-2, Nihon Clea Co., Tokyo) which was supplemented with 0.06% 3′-MeDAB[10] for the experimental group. The control group was fed the same diet without the carcinogen. After 0, 1, 2, 4, 6, 10, 14, 18, and 22 weeks, rat hepatocytes were isolated from rats anesthetized with pentobarbital by collagenase perfusion *in situ*, as described.[11] No significant difference in cell viability (>80%) between hepatocytes from untreated rats and rats treated with 3′-MeDAB from 1 to 14 weeks was found by means of the trypan blue (0.4%) exclusion test. In experimental groups at 18 and 22 weeks, the viability was >60%. Binding experiments with the α_1-adrenergic ligand [^{3}H]prazosin (962.0 GBq/mmol, New England Nuclear) and the α_2-adrenergic ligand [^{3}H]-clonidine (1716.8 GBq/mmol, New England Nuclear) were done in the presence of 10 μM *dl*-phentolamine (Ciba-Geigy) or in its absence on the plasma membranes of rat hepatocytes as previously described.[7] The K_d and B_{max} values for each ligand were calculated by Scatchard analysis. Membrane protein was measured by the method of Lowry *et al.*,[12] with bovine serum albumin as the standard.

The results are shown in Fig. 1. During treatment with 3′-MeDAB, remarkable changes were observed in the numbers of binding sites for both ligands, although there was no significant change in the K_d values for [^{3}H]-prazosin and [^{3}H]clonidine (200 ± 57 pM, n=49 and 18.2 ± 5.2 nM, n=49, respectively) throughout this study. In the control group, there was little change in the binding of these ligands during the study. In experimental groups, the number of α_1-adrenergic receptors, measured in terms of [^{3}H]prazosin binding, decreased to about half of the control level 2 weeks after the start of the diet and this decrease lasted for 22 weeks. On the other hand, an increase of [^{3}H]clonidine-binding sites was observed in hepatocytes from carcinogen-treated rats. The increase was clearly observed at the end of the

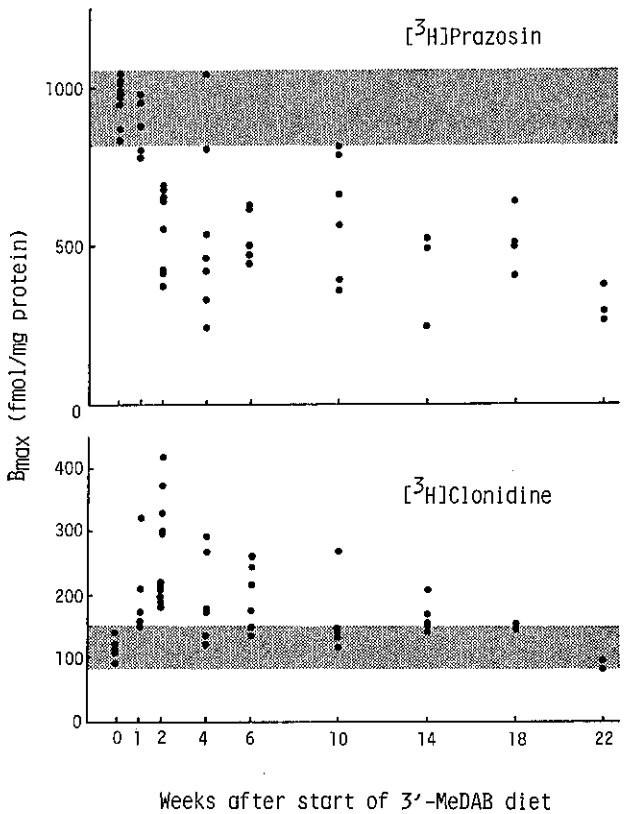

Fig. 1. Changes in binding of [^{3}H]prazosin and [^{3}H]clonidine to hepatic membranes from rats fed 3′-MeDAB. Each point indicates the B_{max} value of [^{3}H]prazosin or [^{3}H]clonidine to hepatic membranes obtained by Scatchard analysis. Dotted zones indicate the range of the B_{max} values of [^{3}H]prazosin and [^{3}H]clonidine in control rats.

first week, and the peak, 3 times the control level, was reached in 2 weeks. Thereafter the increased level declined slowly and had returned to the level of the control group by 18 weeks. The increase in number of α_2-adrenergic receptors in hepatocytes from rats fed 3′-MeDAB was confirmed by the selective action of adrenergic antagonists; [^{3}H]clonidine binding to hepatic membranes from control rats and rats treated with 3′-MeDAB for 2 weeks was inhibited by the α_2-antagonist yohimbine in a dose-dependent manner but was essentially not inhibited by the α_1-antagonist prazosin or the β-antagonist propranolol.

Soon after the start of 3′-MeDAB diet feeding, liver tissue of the experimental rats became yellowish. However, we considered that the binding of this pigment to hepatocytes, which reached a peak at 3 weeks,[6] did not alter the binding of ligands, since 3′-MeDAB had no effect on the binding of [^{3}H]prazosin or [^{3}H]clonidine at the estimated concentration of the carcinogen bound to the hepatocytes.[13]

Three types of adrenergic receptor have been detected in rat hepatocytes: α_1, α_2, and β,[14–16] which are coupled to particular systems of signal transduction; β-adrenergic receptors are coupled to adenylate cyclase in a stimulatory fashion,[14] α_2-adrenergic receptors are coupled to the enzyme in an inhibitory fashion,[17] and α_1-adrenergic receptors are not coupled to adenylate cyclase but are in some way coupled to phosphatidylinositol turnover, and calcium gating and mobilization.[18–20] In adult rats, catecholamines act predominantly through α_1-adrenergic receptors in hepatocytes.[16, 21, 22] In fetal and newborn rat liver, catecholamines appear to act mainly through β-adrenergic receptors.[16, 23] Moreover, fetal rat liver has been reported to be characterized by a large number of α_2-adrenergic receptors, which falls 10-fold by birth.[16] In this study, we found a decrease in the number of α_1-adrenergic receptors and an increase in the number of α_2-adrenergic receptors in rats treated with 3′-MeDAB during the earliest stage of carcinogenesis. Therefore, this is consistent with the hypothesis that during carcinogenesis there emerges a population of cells with fetal characteristics.

(Received March 3, 1990/Accepted April 23, 1990)

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