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Facilitation of liver regeneration after partial hepatectomy by ventromedial hypothalamic lesions in rats

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Abstract. Whether or not the hypothalamus is involved in initiating hepatic DNA synthesis after partial hepatectomy is unclear. To determine the role of the ventromedial hypothalamic nuclei in liver regeneration after partial hepatectomy, we studied hepatic DNA synthesis during liver regeneration in rats with bilateral lesions of these nuclei. Lesions of the ventromedial hypothalamus accelerated the increase in hepatic DNA synthesis and raised the peak level of thymidine incorporation after partial hepatectomy. These effects of hypothalamic lesions were completely inhibited by hepatic vagotomy. Thus, lesions of the ventromedial hypothalamus appear to promote hepatic regeneration by increasing vagal stimulation of the liver.

Key words: Ventromedial hypothalamus – Partial hepatectomy – Liver regeneration – Facilitation

Introduction

Previous studies have suggested that various humoral factors may stimulate DNA synthesis after partial hepatectomy [2, 14, 16]. However, since the autonomic nervous system directly innervates the hepatic parenchyma and has a role in metabolic control [17, 21], it seems likely that liver regeneration may be cooperatively regulated by both humoral factors and the autonomic nervous system. The hypothalamus has a vital role in the integration of neuro-humoral information [18], and it possesses autonomic centres which are connected to the viscera via the autonomic nervous system. We have recently demonstrated that lesions of the ventromedial hypothalamus (VMH) increase hepatic DNA synthesis through the promotion of vagal activity [10]. However, hepatocyte proliferation after partial hepatectomy in rats with VMH lesions has not yet been investigated. To determine whether hepatocyte proliferation after partial hepatectomy was altered by lesions made to the VMH, the present study investigated hepatic DNA synthesis during liver regeneration in VMH-lesioned rats.

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Materials and methods

Animals. Female Sprage-Dawley rats weighing between 230 and 250 g were used. The rats were maintained in cages in a temperature-controlled (23 ± 2° C) animal room with a 12-h light/dark cycle (lights on at 7:00 am). Free access was followed to food and water.

VMH lesions. All surgical procedures were carried out under hexobarbital anaesthesia (50 mg/kg). Lesions of the VMH were performed as described previously [8, 11], with the stereotaxic coordinates for the VMH being the bregma anteriorly, 0.75 mm lateral to the midsagittal line, and 1.0 mm dorsal to the base of the skull according to the atlas of De Groot [5]. A stainless steel needle insulated with Epoxylite (except for 1 mm at the tip) was inserted according to these coordinates and an anodal current of 2 mA was passed through it for 20 s. Sham operations were performed in an identical manner except that no current was applied. After surgery, the rats were returned to their cages and allowed free access to food and water. At the conclusion of the experiment, the brain of each rat was removed, fixed in 10% formalin, and then sectioned and examined microscopically to confirm that the lesions to the VMH had been appropriately made. If the VMH was not destroyed bilaterally, the animal was excluded from the study.

Vagotomy and sympathectomy. Hepatic vagotomy was performed as described previously [22]. The hepatic branch of the vagus nerve leaves the main vagal trunk a few millimeters proximal to the cardia. This branch was exposed and completely transected near the vagal trunk under a dissecting microscope. In the sympathectomized rats, microsurgical hepatic sympathectomy was performed immediately after VMH lesions were made, using the method described previously [7]. All nerves entering the hapatic hilum via the hepato-duodenal ligament were divided and the hepatic branch of the vagus nerve entering the liver close to the hilum was deliberately spared. Sham denervation was achieved by laparotomy without hepatic vagotomy or sympathectomy.

Determination of hepatic DNA synthesis. DNA synthesis was estimated by measuring the incorporation of [methyl-^{3}H]thymidine into DNA. Each rat was given an intravenous injection of (methyl-^{3}H]thymidine (20 μCi/100 g) 1.5 h before being killed by decapita-

Table 1. Food intake (g/day) after VMH lesions or sham lesions

Group	Days after lesions were made						
	1	2	3	4	5	6	7
Sham-lesioned rats	11.7 ± 2.4	12.1 ± 2.0	12.5 ± 1.9	11.4 ± 1.6	12.7 ± 2.5	11.4 ± 2.5	13.1 ± 2.3
VMH-lesioned rats	13.9 ± 2.1	20.8 ± 4.5	27.0 ± 3.6	24.8 ± 4.3	38.1 ± 5.8	34.5 ± 4.8	31.6 ± 4.4

Values are the mean ± SEM, $n = 6$

tion. After sacrifice, the remnant liver was weighed and homogenized in 5% (w/v) trichloroacetic acid using a Polytron homogenizer (Kinematica, Switzerland). The homogenate was then centrifuged twice at 1000 *g* for 1 min, and the precipitate was used for DNA analysis. DNA was extracted with hot 5% trichloroacetic acid (90°C for 20 min) according to the method of Schneider [20], and radioactivity was counted in Aqueous Counting Scintillant II solution (Amersham, USA) using a Beckman scintillation counter (Beckman Instruments, USA). The DNA content was determined by the method of Burton [3].

Experimental procedure

Experiment 1: effect of VMH lesions on DNA synthesis after partial hepatectomy. To determine how VMH lesions influenced hepatic DNA synthesis after partial hepatectomy, the incorporation of [methyl-^{3}H]thymidine into DNA was determined at 0, 12, 18, 24, 30, 36, and 48 h after partial hepatectomy. Immediately after VMH lesions were made, the median and left lateral lobes of the liver were resected by the method of Higgins and Anderson [6]. After partial hepatectomy, the rats were returned to their cages and allowed free access to food and water.

Experiment 2: effect of VMH lesions on restoration of the hepatic DNA content. It is well known that VMH lesions greatly increase the food consumption [1]. To exclude the effect of hyperphagia, we used pair feeding in this experiment. Each of the VMH-lesioned rats was pair fed with a sham-lesioned rat. Food consumption was measured for 7 days after VMH lesions were made by subtracting the residual pellets and spillage from the initial weight distributed to the animals (Table 1). One-third of the daily food allowance was given to each group at 8:00 am and the remainder was given at 8:00 pm. To determine the extent of liver resection, six partially hepatectomized rats were killed immediately after the operation. The excised lobes averaged 68% of the total liver weight, and the preoperative total DNA content of each liver was estimated from the weight and DNA content of the excised tissue on the basis of this proportion. Then the increase of the hepatic DNA content was expressed as a percentage of the estimated preoperative total DNA content.

Experiment 3: effect of hepatic vagotomy or sympathectomy. Four groups of animals with a similar food intake ($n = 6$ each) were prepared: (1) sham-denervated, sham-lesioned rats, (2) sham-denervated, VMH-lesioned rats, (3) VMH-lesioned rats with hepatic vagotomy, and (4) VMH-lesioned rats with hepatic sympathectomy. To eliminate the effect of hyperphagia in VMH-lesioned rats, all the other groups of rats were pair fed with the sham-denervated, sham-lesioned rats. DNA synthesis was estimated 24 h after partial hepatectomy.

Statistical calculations

Results are presented as the mean ± SEM. Statistical comparisons were performed by analysis of variance and Student-s *t*-test, with $P < 0.05$ being taken as the level of significance.

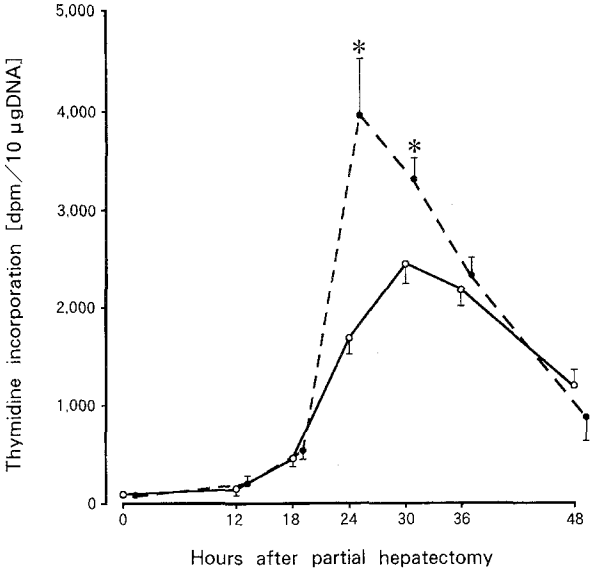

Fig. 1. Effect of ventromedial hypothalamic (VMH) lesions on the incorporation of [methyl-^{3}H]thymidine into hepatic DNA after partial hepatectomy. Each *point* shows the mean ± SEM ($n = 6$). *Solid circles*, VMH-lesioned rats; *open circles*, sham-lesioned rats. * $P < 0.05$ vs sham-lesioned rats

Results

Experiment 1: effect of VMH lesions on DNA synthesis after partial hepatectomy

In sham-lesioned rats, the incorporation of [methyl-^{3}H]-thymidine reached its maximum level at 30 h after partial hepatectomy and subsequently decreased gradually (Fig. 1). In VMH-lesioned rats, incorporation peaked at 24 h and was significantly higher than in sham-VMH-lesioned rats at both 24 and 30 h. The maximum [methyl-^{3}H]thymidine incorporation was also significantly higher in the lesioned rats than in the sham-operated rats (3998 ± 657 versus 2411 ± 110 dpm/10 µg DNA, $P < 0.05$).

Experiment 2: effect of VMH lesions on restoration of the hepatic DNA content

Figure 2 shows the effect of VMH lesions on the restoration of the hepatic DNA content after partial hepatectomy in VMH-lesioned rats which were pair fed with sham-lesioned rats. In VMH-lesioned rats, the percentage restoration of the original hepatic DNA content was

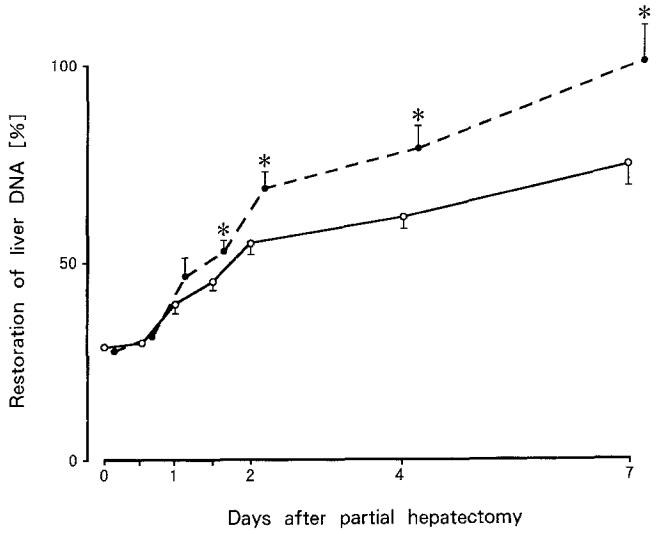

Fig. 2. Effect of VMH lesions on the restoration of hepatic DNA after partial hepatectomy. Each *point* shows the mean ± SEM ($n = 6$). *Solid circles*, VMH-lesioned rats; *open circles*, sham-lesioned rats. * $P < 0.05$ vs sham-lesioned rats

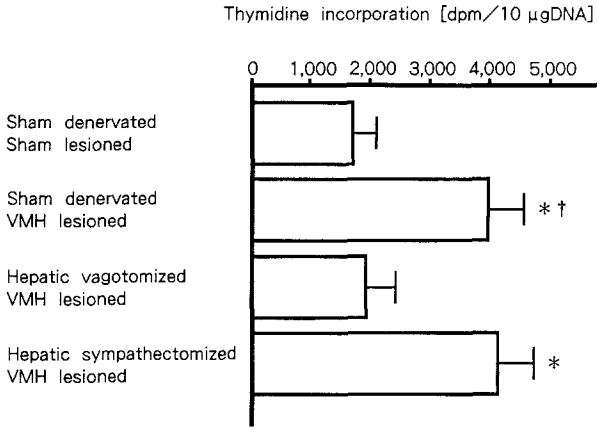

Fig. 3. Effect of hepatic vagotomy or sympathectomy on [methyl-^{3}H]thymidine incorporation at 24 h after partial hepatectomy in VMH-lesioned rats. Each *bar* shows the mean ± SEM ($n = 6$). * $P < 0.05$ vs sham-denervated, sham-lesioned rats. ** $P < 0.05$ for sham-denervated, VMH-lesioned rats vs VMH-lesioned rats with hepatic vagotomy

significantly greater than in sham-lesioned rats at 36 h as well as after 2, 4, and 7 days, and it increased throughout the study period with the progression of liver regeneration.

Experiment 3: effect of hepatic vagotomy or sympathectomy

Thymidine incorporation was significantly decreased in VMH-lesioned rat with hepatic vagotomy compared with that in VMH-lesioned rats with hepatic sympathectomy or sham denervation, and it decreased to the level seen in rats with sham denervation and sham VMH lesions (Fig. 3). There was no significant difference of [methyl-^{3}H]thymidine incorporation between VMH-lesioned rats with hepatic sympathectomy and sham-denervated, VMH-lesioned rats.

Discussion

The present study showed that VMH lesions accelerated the post-hepatectomy increase of hepatic DNA synthesis and increased the peak incorporation of [methyl-^{3}H]-thymidine. This promotion of DNA synthesis by VMH lesions was completely inhibited after hepatic vagotomy, but not after hepatic sympathectomy.

The hepatic parenchyma has been shown to have an abundant parasympathetic innervation [21] and several studies have shown that the hepatic branch of the vagus nerve contains most of the vagal afferents to the liver [19]. Previous studies have suggested that vagotomy influences DNA synthesis during liver regeneration after partial hepatectomy [13, 22]. In addition, we have previously reported that VMH lesions produced an increase in the DNA content of the liver and gastrointestinal tract in rats [9], and that vagal activity produced by VMH lesions stimulated DNA synthesis in those organs [10, 11]. These findings taken together with the results of the present study suggest that the stimulation of vagal activity by VMH lesions might accelerate the increase in hepatic DNA synthesis and raise the peak level of thymidine incorporation after partial hepatectomy.

Nobin et al. [17] have demonstrated the existence of sympathetic innervation of the liver. It has also been suggested that adrenergic agents regulate liver regeneration [15]. Cruise et al. [4] reported that norepinephrine stimulated DNA synthesis by cultured rat hepatocytes through alpha 1-adrenergic receptors. We recently reported that a beta-adrenergic-receptor-mediated mechanism was responsible for the increase of the DNA content in white adipose tissue after VMH lesions were made in rats [12]. In the present study, however, hepatic sympathectomy did not modify the effect of VMH lesions on DNA synthesis after partial hepatectomy. Thus, the hepatic sympathetic nervous system appears to have little facilitatory effect on hepatocyte proliferation.

In conclusion, VMH lesions facilitated hepatic regeneration after partial hepatectomy by a mechanism based on an increase of vagal activity. The present study did not address the receptor and post-receptor mechanisms involved in this phenomenon, so further investigation is required.

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