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Plasma Indocyanine Green Disappearance Induced by Electrical Stimulation of the Hepatic Vagal Branch in Rats

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Summary. **The plasma disappearance curve of indocyanine green (ICG) was estimated for 15 min by a non-invasive serial monitoring system following electrical stimulation of the hepatic vagal branch in anesthetized rats. The stimulation parameters were 6 V, 1 ms width (square wave for 6 min) and 10 Hz. When ICG 0.25 mg/kg (200 μl) was injected into the jugular vein as a bolus, the curve for ICG was obtained; this was shifted downwards by the electrical stimulation of the hepatic vagal branch. The magnitude of ICG response due to stimulation tended to be frequency dependent. No appreciable change in systemic arterial pressure or portal venous blood flow was seen during the ICG estimation.**

These results suggest that the hepatic vagal branch contains signals which directly modify the uptake of ICG by the hepatocytes without hepatic circulatory influence.

Key words—anion transport, hepatic dye metabolism, liver, rat.

INTRODUCTION

The indocyanine green (ICG) test, a method for determining effective hepatic blood flow and hepatocyte function,[1-4] is quite useful for evaluating liver functions, measuring the blood flow in an extrahepatic shunt, and determining the severity of liver disease.[5-7] A non-invasive ICG monitoring system has been developed in which the ICG concentration is expressed serially; its usefulness has been reported in rats and humans.[8-10] On the other hand, the vagal nerve innervating the liver has been shown to terminate in the hepatocytes[11] and participate in the regulation of the liver metabolism.[12-14]

This study was designed to investigate whether eletrical stimulation of the hepatic vagal branch influences plasma ICG disappearance in rats.

MATERIALS AND METHODS

Thirty-six male Wistar rats weighing about 250 g were used. The animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (45 mg/kg), and the depth of anesthesia was maintained by intramuscular injection of the same agent (7.5 mg/kg) every 30 min. Tracheotomy was carried out to provide a patent airway. Throughout the experiments, the rectal temperature was kept at 36.0 $\pm$ 0.6℃ with a heating lamp.

An ICG monitoring system (RK-1000, Sumitomo Electric Industries Ltd., Osaka, Japan) was utilized for this study. Plasma concentrations of ICG were serially plotted between 0 and 15 min after ICG injection. An optical sensor for estimating the ICG concentration was attached to the skin of one hind leg.[10]

Portal venous blood flow (PVF) was measured with a transit-time ultrasonic volume flowmeter (Transonic T201, Advance, NY, USA) connected to a 2 mm probe.[15,16] The systemic arterial blood pressure (SAP) was recorded from the right carotid artery.

ICG (Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan), dissolved in the aqueous solvent provided, was injected into the right jugular vein as a bolus. ICG 0.25 mg/kg (200 μl) was used as a test injection.[10] Atropin sulfate (Tanabe Pharmaceutical Co., Ltd., Tokyo, Japan) dissolved in physiological saline (100 μl) was administered intramuscularly 30 min before ICG injection.

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The elastic electrode for nerve stimulation[17] was set on the caudal cut end of the hepatic vagal branch, while the sympathetic hepatic branch remained intact. The stimulation parameters were 6 V, 1 ms width (square wave for 6 min) and the frequencies were 1, 5, 10 and 20 Hz.

The data were ANOVA analyzed and specific values were evaluated by Duncan's test. A value of $p<0.05$ was regarded as significant.

RESULTS

The plasma disappearance curves for ICG are shown in Fig. 1. The curve shifted downwards as the hepatic vagal branch was stimulated by electricity (6 V, 1 ms width square wave and 10 Hz for 6 min). A stimulating effect on the ICG concentration appeared about 10-15 min later and the intensity of the response tended to be frequency dependent in the range of 1 to 20 Hz, as shown in Fig. 2. Sectioning of the hepatic vagal branch at a level immediately below the exciting electrode abolished such responses to electrical stimulation, indicating that these responses could be ascribed to a change in peripheral nervous excitation (Fig. 2). These ICG responses were provoked without any change in SAP or PVF (Fig. 3). Prior administration of atropin sulfate (0.5 mg/kg) failed to change ICG response caused by hepatic vagal stimulation (Table 1).

DISCUSSION

We found that the hepatic vagal nerve moderates ICG dynamics; electrical stimulation applied to the hepatic vagal branch could be regarded as enhanced efferent vagal activity.

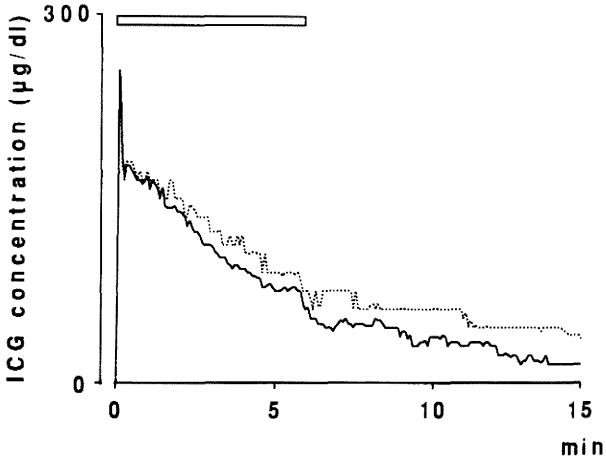

Fig. 1. Representative alterations in plasma disappearance curves of ICG following electrical stimulation of the hepatic vagal branch. ICG 0.25 mg/kg was administered with (—) or without (···) electrical stimulation (10 Hz). Zero indicates the time of ICG injection, and the rectangle the time of stimulation.

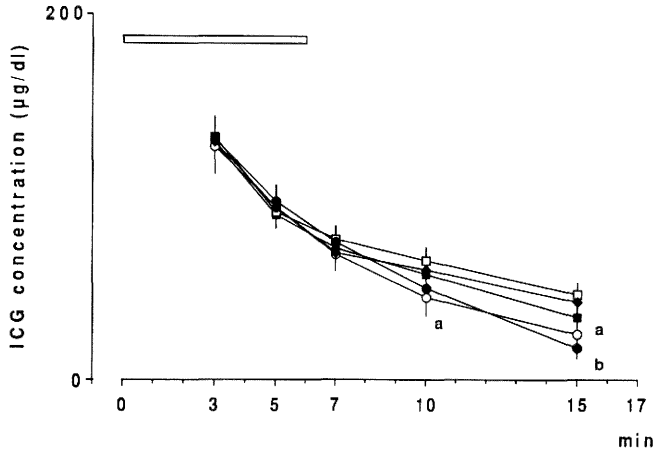

Fig. 2. Plasma disappearance curves of ICG caused by hepatic vagal stimulation. Four different frequencies of electrical stimulation (□, 1 Hz; ■, 5 Hz; ○, 10 Hz; ●, 20 Hz; ◆, 20 Hz with sectioning of the nerve below the exciting electrode) are given. Zero shows the time of ICG injection, and the rectangle the time of stimulation. Values are the means ± SEM ($n=6$). $^{a}p<0.05$ vs □ and ◆. $^{b}p<0.01$ vs □ and ◆

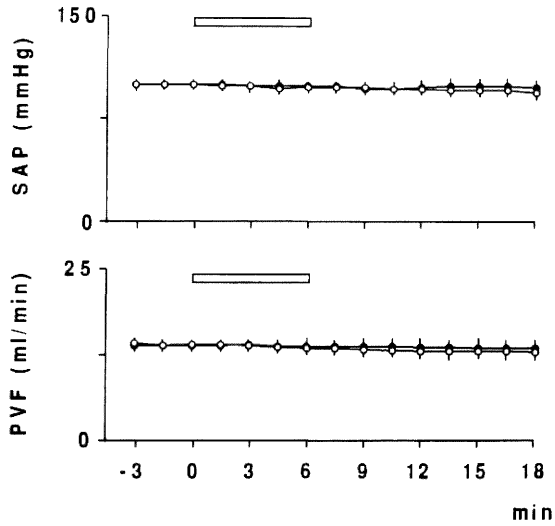

Fig. 3. Changes in SAP and PVF after hepatic vagal stimulation. ICG 0.25 mg/kg was injected with (○) or without (●) stimulation (20 Hz). Zero indicates the time of ICG injection, and the rectangle the time of stimulation. Values are the means ± SEM (n=6).

Table 1 ICG concentrations after hepatic vagal stimulation with (I) or without (II) atropin

	6 min later (μg/dl)	15 min later (μg/dl)
I	76.0 ± 7.5	33.4 ± 11.0
II	88.6 ± 8.2	45.4 ± 10.2

Values are the means ± SEM (n=6).

Because the disappearance of ICG in the plasma due to vagal stimulation tended to be electrically frequency dependent, the ICG response may be mediated by neural excitation.

ICG dynamics have been shown to correlate with hepatic blood flow or hepatocyte function or both.[1-4] In the present study, hepatic vagal stimulation did not cause any change in portal venous circulation. In connection with this, it has been noted that the hepatic arterial blood flow is unaffected by hepatic vagal stimulation in rats (Ohtake & Sakaguchi, unpublished data). It is therefore believed that the ICG response observed originated in the hepatocyte function.

Once ICG enters the liver, it does not backflow into the plasma, and the uptake of the dye by the hepatocytes has been estimated to take 20 min.[18-20] Considering these reports together with the finding that the neural stimulating effect on ICG appeared in about 10 min, it is possible that the vagal nerve accelerates the uptake of ICG by the hepatocytes.

Although the dose of atropin used in this study has been shown to block cholinergic effect of the vagal nerve,[21] the agent did not change ICG response caused by hepatic vagal stimulation. This could mean that there is no cholinergic mechanism in the phenomena.

ICG transport mechanisms across the hepatocyte membrane have been reported,[3,22] but the neural factor was not involved in the previously proposed explanation. Concerning this, it has been shown that neural electrical activity moderates anion transport across the biological membrane.[23,24] This may hold true for the observed ICG response.

Carbohydrate metabolic components have been shown to modify efferent activity of the hepatic vagal branch.[21,25] Further study in the situations mentioned above will be necessary to define the vagal function on ICG movement.

These observations lead us to conclude that the hepatic vagal branch contains signals which directly moderate ICG dynamics in the liver.

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