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RAPID COMMUNICATION

EVIDENCE FOR THE ASSOCIATION BETWEEN TOTAL HEPATIC BLOOD FLOW AND HEPATIC GLUTATHIONE CONTENT

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Glutathione is a ubiquitous tripeptide which has an important role in many redox and detoxification reactions (1,2). The liver is the most important organ involved in the biosynthesis and interorgan transport of GSH (3,4). In view of the central role of the liver in GSH metabolism, it is reasonable to expect that the liver should be able to rapidly increase the rate of GSH biosynthesis when faced with an oxidative stress resulting in the depletion of GSH. In order to increase the rate of GSH biosynthesis, it would be necessary to increase the supply of substrates, in particular L-cysteine [the rate-limiting amino acid in GSH biosynthesis (4)], to the liver. One mechanism whereby an increased rate of substrate delivery could be accomplished is by an increase in liver blood flow. In this paper, we have examined the hypothesis that total hepatic blood flow (tHBF) is sensitive to hepatic GSH content. The thermal pulse decay (TPD) method (5,6) was used to assess local tHBF in anesthetized rats. Diethylmaleate (DE) was used to rapidly deplete hepatic GSH (7), while L-cysteine (CY) was used to partially restore hepatic GSH in previously GSH-depleted rats.

MATERIALS AND METHODS

In the TPD method, a 3-sec power pulse is applied to heat thermistor microprobes embedded in the tissue. Subsequent to the heating pulse, the embedded microprobes serve to measure the local tissue temperature decay. A computer-assisted data acquisition system is used to sample (10/sec) the temperature decay. Calculation of the local tissue blood flow is based on a "best-fit" comparison of the measured decay to that predicted by the bioheat equation (5). Each probe used in the TDP method evaluates the local tissue blood flow. In the case of the liver, tissue blood flow includes contributions from both arterial and portal venous sources. This fact is reflected in the concept that, in the liver, the TDP method measures the local tHBF.

Male Sprague-Dawley rats (~500 g, Harlan Sprague-Dawley Inc., Indianapolis, IN) were anesthetized by an i.p. injection of sodium pentobarbital (40 mg/kg). The liver was exposed via an abdominal incision parallel to the costal margin on the right side, and four

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to six thermistor microprobes were inserted into the liver at various locations. The incision was closed, and the animals were allowed to stabilize for at least 20 min before measurements were made. Body temperature, as measured by the liver-embedded microprobes, was maintained between 37 and 38° with a heating pad throughout the experiment.

After the stabilization period, tHBF was measured at 3-min intervals during each 2-hr experimental trial. Six rats were used in Study 1. After obtaining measurements during a 30-min control period, the animals were injected i.p. with DE (0.1 ml/100 g). Forty-five minutes later, the rats were injected i.p. with CY (20 mg/100 g in 0.9% saline), and tHBF was monitored for another 45-min period. At the end of each trial, the animal was killed by an overdose of sodium pentobarbital.

In Study 2, five rats were subjected to a similar time-treatment protocol except that CY treatment preceded the treatment with DE.

TDP perfusion measurements, made in four to six different liver locations in each animal during the last 15 min of each experimental treatment period, were pooled and subjected to analysis of variance with unequal means. A P value of < 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Control tHBF values (ml x g^{-1} x min^{-1}, mean ± SEM) in Studies 1 and 2 were 2.1 ± 0.1 and 2.2 ± 0.1 respectively. These values were virtually identical to the previously reported tHBF of 1.9 ± 0.1 ml x g^{-1} x min^{-1} (8).

Hepatic GSH content in response to the various treatments could not be determined in the present study because the removal of a portion of the liver would have induced considerable trauma and hemorrhage. However, the effects of DE and CY on hepatic GSH are well documented (4,7). Additionally, we have examined changes in hepatic GSH content in a separate study using rats subjected to a similar time-treatment protocol (9). In that study, control hepatic GSH content (μmoles/g liver) was 6.0 ± 0.6 (mean ± SEM, N = 5). DE treatment depleted hepatic GSH to 10% of control values (0.6 ± 0.1, N = 5), while CY treatment of DE-treated rats partially restored hepatic GSH content (1.4 ± 0.3, N = 4). All values of hepatic GSH content were significantly different from each other.

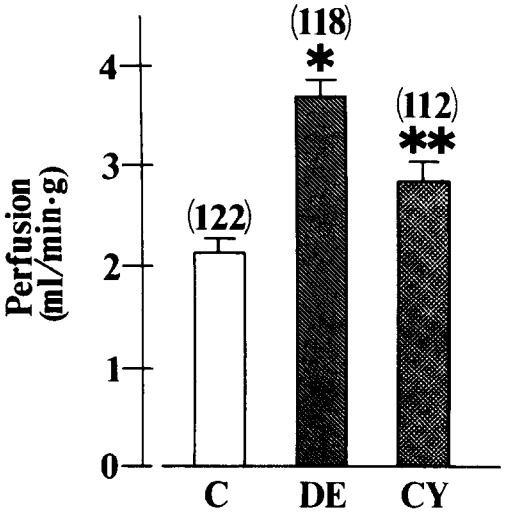

Fig. 1. tHBF (PERFUSION; ml/min x g) in anesthetized rats during a control (C) period and subsequent i.p. injections of diethylmaleate (DE) and L-cysteine (CY). Values shown are the mean ± SEM of the number of observations shown in parentheses obtained in six rats.
*,**Significantly different from C and each other, $P < 0.01$.

Figure 1 summarizes the results of Study 1. DE treatment was associated with a significant ($P < 0.01$) increase in tHBF relative to that in control condition. As mentioned above, hepatic GSH in DE-treated rats was approximately 10% of controls. CY treatment of DE-treated rats was associated with a significant ($P < 0.01$) reduction in tHBF, but it was still significantly ($P < 0.01$) higher than that in the control condition. Since CY treatment of DE-treated rats only partially restores hepatic GSH content (9), then the above results are consistent with the hypothesis that tHBF is related to hepatic GSH content.

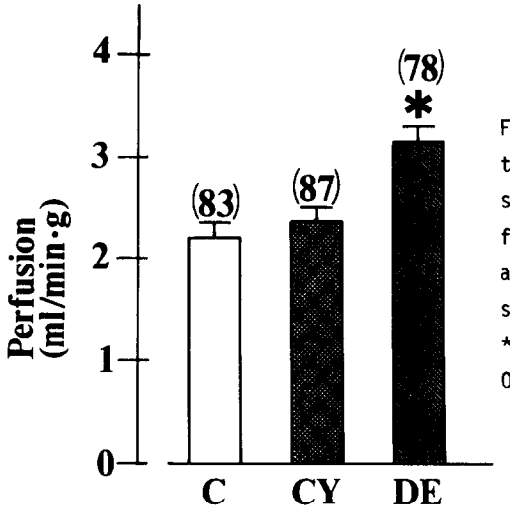

Fig. 2. tHBF (PERFUSION; ml/min x g) in anesthetized rats during a control (C) period and subsequent i.p. injections of L-cysteine (CY), followed by diethylmaleate (DE). Values shown are mean ± SEM of the number of observations shown in parentheses obtained from five rats. *Significantly different from C and CY, $P < 0.01$.

It may be argued that DE and CY had nonspecific effects on tHBF that were not related to hepatic GSH content. However, the results of Study 2 (Fig. 2) contradict this argument. CY treatment of control rats was not associated with any changes in tHBF. This would not be expected if CY had nonspecific effects on tHBF. On the other hand, the above results would be expected if the effect of CY on tHBF was mediated via changes in hepatic GSH content since CY treatment of GSH-replete rats does not result in an enhancement of hepatic GSH content (4). However, as shown in Fig. 2, the subsequent treatment with DE did result in a significant ($P < 0.01$) increase in tHBF and, interestingly, the absolute value of tHBF attained was similar to that attained following CY treatment of DE-treated rats in Study 1 (Fig. 1).

The above results, taken together, provide strong evidence in support of the hypothesis that tHBF may be sensitive to hepatic GSH content. The precise mechanisms linking changes in hepatic GSH content to changes in tHBF are not known. However, it is known that prostaglandin biosynthetic pathways are affected by GSH levels (10,11). Therefore, in the present study, changes in GSH levels may have induced changes in prostaglandin metabolism, which in turn produced the observed changes in tHBF. In addition, it is also not known which of the two sources (hepatic artery and portal vein) of tHBF is changing in response to changes in hepatic GSH content. Clearly, further work is required to fully understand the basis of this interesting relationship between tHBF and hepatic GSH content.

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