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Splanchnic uptake of haloperidol and release of reduced haloperidol *in vivo* in the guinea pig

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Abstract—Splanchnic uptake of haloperidol (HAL) and release of reduced haloperidol (RHAL) were studied *in vivo* in guinea pigs. Anesthetized animals with implanted cannulae in the aorta, the hepatic vein and the inferior vena cava were infused intravenously with HAL at a rate of 9.6 μg/min/animal for 90 min. Plasma HAL and RHAL in samples taken from the arterial and hepatic venous cannulae were measured by HPLC with an electrochemical detector. Contamination of the hepatic venous samples by blood from the inferior vena cava was ruled out by the validation method of tritiated water washout [Huang MT, *J Appl Physiol* **71**: 359–364, 1991]. HAL concentrations plateaued at 70 ~ 80 ng/mL in the aorta and 5 ~ 7 ng/mL in the hepatic vein during the final 30 min of infusion. Splanchnic extraction of HAL was 91%. Hepatic blood flow was estimated to be 1.95 ± 0.40 (SD) mL/min/g. If assuming that splanchnic uptake of HAL took place in the liver, a rate of uptake of HAL in the liver of 79.2 ± 18.6 (SD) ng/min/g could be calculated by the Fick principle. The uptake in the whole liver accounted for 14% of the rate of HAL infusion into the animal. Plasma RHAL in the aorta, 6.4 ± 6.6 (SD) ng/mL at 60 min and 9.4 ± 4.6 (SD) ng/mL at the end of HAL infusion, was about 10–12-fold less than aortic HAL. The concentrations of RHAL in the hepatic vein were not significantly different from those in the aorta, indicating that splanchnic tissues including the liver are not responsible for plasma RHAL secretion. The highly efficient uptake of HAL as well as the ketone reductases found previously *in vitro* in liver microsomes of guinea pigs were probably involved only in biliary excretion of HAL.

Haloperidol (HAL*) 4-[4-(*p*-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone, is a potent antipsychotic and antimaniac drug [1]. In humans, HAL can be metabolized and in the process it loses some of its potency through the reduction of the ketone in the butyrophenone moiety of the drug to an alcohol [2–4]. The product of the reductive reaction, known as reduced haloperidol (RHAL), can be re-converted back to HAL and thus regain therapeutic potency in humans [5]. The pharmacokinetics of HAL and RHAL are important determinants of the efficacy of HAL treatment [6]. It was reported that the response to HAL treatment is related inversely to the level of plasma RHAL [7], and that the treatment is effective only within an optimal range of plasma RHAL/HAL ratios in schizophrenic patients [7, 8]. Notwithstanding their importance, clinical pharmacokinetics of HAL and RHAL have been complicated by inter-subject [9] and inter-racial variations [8, 10].

The metabolism of HAL and RHAL in experimental animals also exhibits species difference. Guinea pigs were thought to be a suitable animal model for unraveling the metabolic origin of RHAL, because this species was in common with humans in its ability to inter-convert RHAL and HAL [11–13]. Rats are different from humans and guinea pigs in that they can only convert RHAL to HAL, but not the reverse [12]. Although a ketone reductase system has been identified *in vitro* in liver microsomes of guinea pigs [12], the precise role of the liver in the exchange of HAL *in vivo* in either humans or guinea pigs has not been determined. In the present study, splanchnic uptake of HAL and release of RHAL during an intravenous infusion of HAL were determined by arterio-venous difference technique from the concentrations of HAL and RHAL in the aorta and the hepatic vein.

Materials and Methods

Guinea pigs of both sexes (400–500 g) were anesthetized with an intraperitoneal injection of ketamine (250 mg/kg). Through a ventral incision in the neck, polyethylene cannulae (Intramedic PE 10; Clay Adams), each measuring 60 cm, were implanted in the aorta through the common carotid artery, in the hepatic vein and the abdominal inferior vena cava through the right external jugular vein [14]. The cannula for the abdominal inferior vena cava was inserted 11 cm, and that for the hepatic vein was inserted 7.5 cm from the point of entry in the external jugular vein. The cannula in the abdominal inferior vena cava was used for infusion or injection, and those in the hepatic vein and the aorta were for blood sampling. Following cannulation, the animals were placed on a thermal blanket (Harvard Apparatus). Normothermia at 37° was maintained throughout the experiment. The animals were kept in the anesthetized state during the course of experiments with occasional injections of ketamine as needed.

Since sampling of pure hepatic venous blood is crucial to the current study, the possibility of vena caval contamination in the hepatic venous cannula was carefully tested by validation methods of hepatic extraction of sulfbromophthalein (BSP) and tritiated water washout developed recently in our laboratory [14]. BSP was infused, while tritiated water was injected in a bolus into the abdominal inferior vena cava. Since both must perfuse first to the systemic circulation and then through the liver to the hepatic vein, a correctly implanted hepatic venous cannula is indicated by a steady and significant splanchnic extraction of BSP and by a pattern of tritiated water washout indicative of systemic dilution. To accommodate the validation tests, the experiments were divided into three segments, consisting of two BSP infusions and a bracketed period of HAL infusion.

BSP was infused intravenously at a rate of 0.16 mg/min by an infusion pump (Harvard Apparatus) through the cannula implanted in the inferior vena cava for 60 min. Four simultaneous sets of blood samples (0.2 mL) were taken manually from the cannulae implanted in the aorta and the hepatic vein into attached syringes every 10 min during the final 30 min of infusion. As was found previously in the rat, plasma concentrations of BSP were at a plateau during this period of infusion [14]. Plasma samples were mixed in 0.05 N NaOH, and the concentrations of BSP were measured spectrophotometrically at 578 nm.

* Abbreviations: HAL, haloperidol; RHAL, reduced haloperidol; BSP, sulfbromophthalein.

Splanchnic extraction of BSP was calculated by dividing the concentration difference of BSP in the aorta and the hepatic vein by the aortic concentration of BSP [14]. The clearance of BSP in the animal was calculated by dividing the rate of infusion by the aortic concentration of BSP. Since the liver and no other organ in the splanchnic bed is responsible for BSP metabolism in the animal, the concentrations of BSP in the aorta and the portal vein were expected to be equal. Total hepatic blood flow can be calculated by dividing the clearance of BSP by the splanchnic extraction of BSP and volume fraction of blood plasma [15].

Thirty minutes were allowed to clear remaining BSP in the body at the end of the first BSP infusion. Subsequently, intravenous infusion of HAL lasting for 90 min at a rate of 9.6 μg/min into the abdominal inferior vena cava was initiated. Four sets of blood samples were again taken at 10-min intervals from the arterial and the hepatic venous cannulae during the final 30 min of infusion. Plasma concentrations of HAL, RHAL and chlorohaloperidol, which was added as an internal standard, were measured by HPLC with an electrochemical detector [16]. BSP infusion was repeated 30 min after HAL infusion to detect any possible dislocation of the cannula during the course of HAL experiment. Plateau concentrations of BSP in the aorta or the hepatic vein were not significantly different between the first and the second BSP infusion, indicating that the metabolic state of the liver was not altered by the series of infusions. To prevent any reduction in blood volume, blood taken from the animal was replaced immediately by transfusion of an equal volume of blood from donor animals.

Validation by tritiated water washout was carried out at the conclusion of the second BSP infusion [14]. A 180-cm piece of Tygon tubing (i.d. 0.01 inch; Fischer Scientific) was connected to the hepatic venous cannula for storing serial samples to be withdrawn from the cannula. The selection of the thinnest tubing minimized streaming and mixing of the sequential samples [14]. A bolus of tritiated water (50 μCi/100 μL) was injected into the abdominal inferior vena cava, withdrawing of blood by a syringe into the storing tubing was started concurrently with the injection and was continued thereafter for a total of 3 min. The Tygon tubing was finally cut into 12 equal sections. Since the first three samples consisted of pre-filled saline in the cannula, the fourth data point was selected as zero time of the time-course. The time-course of the washout of the injected tritiated water in the cannula was expressed by counts per minute (cpm) in each section of the cut Tygon tubing.

HAL, RHAL and chlorohaloperidol were obtained from Janssen Pharmaceutic (Beerse, Belgium). BSP was obtained from Sigma. The significance of the difference between the concentrations of HAL and RHAL in the arterial and venous samples was tested by paired *t*-test.

Results and Discussion

All reported data were derived from animals with correctly implanted hepatic venous cannulae in compliance with criteria delineated previously for the validation of tritiated water washout [14]. Typical patterns of the washout of tritiated water from a correctly and an incorrectly implanted cannula are shown in Fig. 1. Radioactivities in the sequential samples from all the correctly cannulated guinea pigs increased gradually and peaked 2 min after tracer injection (Fig. 1), as was previously reported for rats [14]. In animals with incorrectly implanted cannulae, radioactivities in the sequential samples increased abruptly and peaked within the first minute of tracer injection (Fig. 1).

Blood samples obtained from correctly implanted cannulae showed significant extraction of BSP. Both the aortic and hepatic venous BSP remained steady during the

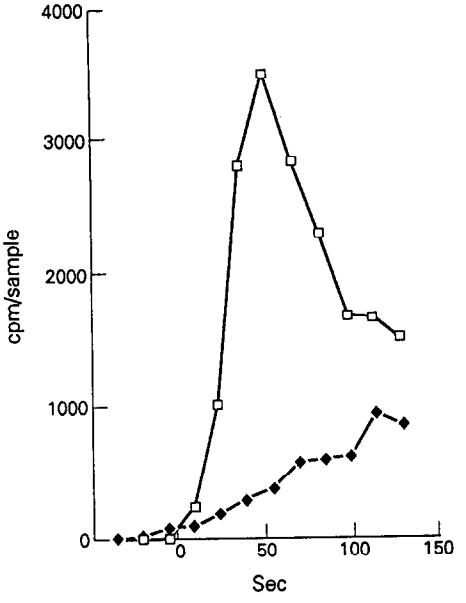

Fig. 1. Time-courses of the washout of tritiated water in a correctly implanted (■) and an incorrectly implanted hepatic venous cannula (□). Data from representative experiments were shown. The procedure for bolus injection of tritiated water into the abdominal inferior vena cava (50 μCi/0.1 mL) and withdrawing of sequential blood samples from the cannula implanted in the hepatic vein were as described in Materials and Methods.

two periods of BSP infusion. Mean plateau concentration of BSP in the aorta was 18.1 ± 2.2 (SD) μg/mL and in the hepatic vein was 10.0 ± 2.9 (SD) μg/mL. Splanchnic extraction of BSP was 0.45 ± 0.10 (SD), which is not significantly different from the value found previously for conscious rats [14]. Total hepatic blood flow determined from splanchnic extraction and the clearance of BSP was 1.95 ± 0.40 (SD) mL/min/g.

The concentrations of HAL were 70 ~ 80 ng/mL in the aorta and 5 ~ 7 ng/mL in the hepatic vein during the final 30 min of HAL infusion (Table 1). The differences between successive samples in either the aortic or the hepatic venous group were not significant, indicating that plasma concentrations of HAL were at a plateau. The concentration of HAL in the aorta was about 10 times greater than that in the hepatic vein, indicating a large first-pass extraction of HAL (91%) in the splanchnic tissues.

The extraction of HAL in the splanchnic tissues is likely to occur exclusively in the liver, since the organ is generally considered to be the major site for drug metabolism. Under such circumstance, the Fick principle can be applied to calculate the rate of uptake of HAL in the liver from the splanchnic gradient of HAL and total hepatic plasma flow. The average rate of hepatic HAL uptake was estimated to be 79.2 ng/min/g wet weight and 1.36 μg/min for the whole liver. It can be estimated that the liver extracts about 14% of the HAL infused into the animal. Since the splanchnic extraction of HAL was 91%, it can be further estimated that the fraction of the systemic circulation or cardiac output perfused to the liver is about 17% in guinea pigs in the anesthetized state.

The concentrations of aortic RHAL, ranging from 6 to 9 ng/mL, were about 10–12-fold less those of aortic HAL (Table 2). RHAL concentrations in the hepatic vein, ranging from 3 to 14 ng/mL, were not significantly different

Table 1. Plasma HAL concentrations in the aorta and the hepatic vein of the guinea pig during a constant intravenous infusion of HAL at a rate of of 9.6 μg/min/animal

	Time of infusion (min)			
	60	70	80	90
Aorta	70.2 ± 3.0	75.6 ± 8.8	73.9 ± 11.2	80.2 ± 5.5
Hepatic vein	7.1 ± 2.9	5.3 ± 1.4	7.2 ± 2.0	7.4 ± 2.0
P*	<0.01	<0.01	<0.01	<0.01

Units are ng/mL. Data are means ± SD of four experiments.
* Significance of difference between arterial and venous samples by paired *t*-test.

Table 2. Plasma concentrations of RHAL in the aorta and the hepatic vein of the guinea pig during constant intravenous infusion of HAL at a rate of of 9.6 μg/min/animal

	Time of infusion (min)			
	60	70	80	90
Aorta	6.4 ± 6.6	7.2 ± 4.0	8.6 ± 5.4	9.4 ± 4.6
Hepatic vein	3.2 ± 1.5	5.8 ± 2.3	9.4 ± 3.8	13.8 ± 7.0
P*	<0.1	<0.1	<0.1	<0.1

Units are ng/mL. Data are means ± SD of four experiments.
* Significance of difference between arterial and venous samples by paired *t*-test.

from the respective RHAL concentrations in the aorta (Table 2), indicating that the splanchnic bed was not secreting plasma RHAL. Although RHAL concentrations in both the aorta and the hepatic vein tended to increase gradually, the differences between successive samples were not statistically different (Table 2).

The sites of origin of plasma RHAL were not clear in the present study. Since ketone reductases have been detected in the cytosol of nearly every mammalian tissues [17], plasma RHAL could originate from any extrahepatic tissue known to contain this enzyme. Current results, nevertheless, allow the conclusion that liver reductases including the microsomal enzyme reported by Korpi *et al.* [12] in guinea pig livers are not involved in the secretion of plasma RHAL *in vivo*. These liver enzymes may be involved in the formation of RHAL glucuronides for biliary excretion [18].

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The suppression of hepatic cytochrome P4504A mRNA mediated by the interferon inducer polyinosinic acid·polycytidylic acid

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Abstract—Interferon and interferon inducers are well known to depress the cytochrome P450-dependent hepatic mixed-function oxidase system and cause a decrease in the capacity of the liver to metabolize drugs and xenobiotics. In this study we have shown that the interferon-mediated changes in an induced form of hepatic cytochrome P450 (CYP4A) are mediated via a depression in the levels of mRNA as assessed by Northern blot and slot blot analyses using a 20-base synthetic oligodeoxyribonucleotide hybridization probe. Rats were pretreated with clofibrate to maximize CYP4A mRNA levels prior to the administration of polyinosinic acid·polycytidylic acid (poly IC), an α/β interferon inducer. Hepatic CYP4A mRNA levels were decreased by 49 and 30% at 6 and 24 hr, respectively, following poly IC administration. In hepatic microsomes cytochome P450 and functional CYP4A as measured by lauric acid hydroxylation, were not affected at 6 hr, but were depressed by 39 and 27%, respectively, 24 hr following poly IC administration. These results suggest that interferon depresses induced levels of hepatic drug metabolism by lowering the level of cytochrome P450 mRNAs and subsequent synthesis of cytochrome P450 apoproteins.

In 1976, two laboratories [1, 2] simultaneously reported that the α/β interferon inducer tilorone depressed hepatic cytochrome P450 and related biotransformation. Subsequently it was shown that twelve different α/β interferon inducers, representing a wide variety of structures and molecular weights, depressed the microsomal cytochrome P450 system in rats and it was suggested that cytochrome P450 depression was a general property of interferon inducers [3]. Singh *et al.* [4] first reported that recombinant human α interferon depressed cytochrome P450 in mice and this has been confirmed for α, β and λ interferon [5–7]. It appears that the action of these recombinant interferons on cytochrome P450 is inseparable from the antiviral and antitumour actions [5, 8, 9]. Although it has been suggested that the loss in cytochrome P450 results from a loss in heme synthesis [10], several studies have now suggested that interferon or its inducers can depress the levels of constitutive hepatic microsomal cytochrome P450 by decreasing the synthesis of the cytochrome P450 apoprotein [11–15]. To date it appears that the synthesis and expression of induced forms of cytochrome P450 are resistant to this effect.

In this study we have shown, using a synthetic oligodeoxyribonucleotide hybridization probe, that the level of hepatic mRNA coding for the induced cytochrome P450 form CYP4A is decreased in clofibrate-treated rats receiving the α/β interferon inducer polyinosinic acid·polycytidylic acid (poly IC). This demonstrates that the interferon-mediated decrease of an induced form of cytochrome P450 is a pre-translational event resulting in a decrease in the synthesis of the cytochrome P450 apoprotein.

Materials and Methods

Bovine serum albumin, clofibrate, Ficoll (type 400), formamide, guanidine-HCl, lambda DNA Hind III digest, 3-[*N*-morpholino]propanesulfonic acid, polyvinylpyrrolidone (PVP-360), and salmon sperm DNA were purchased from the Sigma Chemical Co., St. Louis, MO. Spermidine trihydrochloride was purchased from the Aldrich Chemical Co. Inc., Milwaukee, WI. Poly IC was purchased from Calbiochem, La Jolla, CA. T4 polynucleotide kinase was purchased from Pharmacia Inc., Dorval, Quebec. Agarose was obtained from Bio-Rad Laboratories Ltd., Mississauga, Ontario. GeneScreenPlus® hybridization transfer membrane was purchased from Du Pont Canada Inc., Dorval, Quebec. [^{32}P]ATP was purchased from either ICN Biomedicals Canada Ltd., Montreal, Quebec (7000 Ci/mmol) or Dupont Canada Inc., Dorval, Quebec (6000 Ci/mmol). [1-^{14}C]Lauric acid (10–30 mCi/mmol) was purchased from Amersham Canada Ltd., Oakville, Ontario.

Male Sprague–Dawley rats (200–350 g) obtained from Canadian Hybrid Farms, Kentville, N.S., were housed in wire-bottom cages and fed Purina rat chow and water *ad lib*. They were allowed to acclimatize in our facilities for at least 4 days before receiving drug treatment. In experiments designed to assess the effects of poly IC, rats were induced with clofibrate (250 mg/kg in corn oil) i.p. for 4 days to increase CYP4A and its mRNA [6]. They were killed 24 hr after the last clofibrate injection. Poly IC (10 mg/kg in saline) or an equivalent volume of saline was given i.p. to clofibrate-induced rats 6 or 24 hr before they were killed.

Previously described methods were used for the preparation of hepatic microsomes [17] and for the determination of microsomal protein [18], cytochrome P450 and lauric acid hydroxylation [20]. A portion of the liver was removed for microsome preparation and the remainder was quick frozen in liquid nitrogen prior to the isolation of RNA using the method outlined by Protter *et al.* [21]. All RNA samples prepared had a 260 to 280 nm absorbance ratio of greater than 1.9.

The sequence used for the oligomer was based on the rat cDNA sequence for CYP4A published by Hardwick *et*