Fatigue of Cholestasis and the Serotoninergic Neurotransmitter System in the Rat

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Fatigue associated with cholestasis may impair health-related quality of life. The pathogenesis of this symptom is largely unknown, but it has been suggested that central serotoninergic neurotransmission may be implicated and that serotonin 1A receptor agonists may yield improvement. The aim of this study was to study the central serotoninergic system, specifically the serotonin (5-HT)$_{1A}$ receptor–mediated pathway of serotoninergic neurotransmission, in a bile duct resection rat model of cholestasis. Fatigue was assessed in the forced swim test in sham and bile duct–resected rats. The serotonin behavioral syndrome, which includes hyperlocomotion, was assessed in both groups of rats after escalating doses of the 5-HT$_{1A}$ receptor agonist 8-hydroxy(di-n-propylamine)tetralin (8-OH DPAT). 5-HT$_{1A}$ and 5-HT$_2$ receptor densities were explored in four brain regions using a receptor-binding assay. Extracellular 5-HT and 5-hydroxyindoleacetic acid were measured via *in vivo* brain dialysis. Bile duct–resected rats spent more time floating in the forced swim test, and 8-OH DPAT decreased floating time in cholestatic rats ($P < .01$). Dose–response curves created with 8-OH DPAT for the serotonin behavioral syndrome were similar in bile duct–resected and sham-operated rats. 5-HT$_{1A}$ and 5-HT$_2$ receptor densities in most brain regions and extracellular serotonin levels were similar in both groups of rats. In conclusion, 5-HT$_{1A}$ receptor agonist–induced amelioration of fatigue in cholestatic rats may be nonspecific and not linked to reversal of the pathophysiology of fatigue associated with cholestasis; however, these data do not exclude a potential role of the central serotoninergic system in the evolution of fatigue. (HEPATOLOGY 2005;41:731-737.)

Fatigue is an intriguing symptom because of its subjective nature and the inherent difficulty of its assessment.[1] It is important to differentiate between the fatigue that occurs in a patient with a terminal illness (*e.g.*, the late phases of cancer) and the fatigue that occurs in a patient with a "less severe" disease. Although fatigue in the former condition is easy to explain, the fatigue of less severe disease has been neglected for a long time. Fatigue occurring in patients with liver disease such as chronic viral hepatitis or cholestatic liver disease falls into this category. Fatigue in these conditions appears to impair health-related quality of life.[2,3] Its pathogenesis is unknown. It is possible that the pathogenesis may differ from condition to condition[4]; for example, in chronic hepatitis C, the hepatitis C virus may be directly linked to the pathophysiology leading to fatigue,[5] whereas in cholestatic liver disease the pathogenesis continues to be an enigma.[6]

Mounting evidence suggests that fatigue occurring in patients with cholestatic liver disease is of central and not peripheral origin.[7,8] Several functions of the central nervous system have been suggested to contribute to the genesis of fatigue in cholestasis: (1) the defective release of corticotrophin-releasing hormone,[8-10] (2) an imbalance of manganese homeostasis in the brain,[4] and (3) altered serotoninergic neurotransmission.[11,12] The latter hypothesis emerged largely from the findings of a study performed in rats with cholestasis secondary to bile duct ligation. In this study, a serotonin (5-HT)$_{1A}$ receptor agonist was reported to improve fatigue in a swim test model

Abbreviations: 5-HT, serotonin; 8-OH DPAT, 8-hydroxy(di-n-propylamine)tetralin; BDR, bile duct resection; 5-HIAA, 5-hydroxyindole acetic acid.

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used to assess fatigue.[11] Based on this study, the authors suggested that enhancement of central 5-HT_{1A} receptor–mediated neurotransmission may be of benefit to patients with fatigue associated with cholestasis. The results of this study have not been confirmed nor refuted thus far. Because 5-HT_{1A} receptor agonists are known to induce the serotonin behavioral syndrome, which consists of hyperlocomotion (circling), flat body posture, reciprocal forepaw treading, and head weaving,[13,14] the possibility exists that the increase in motor activity detected during the forced swim test in cholestatic rats after administration of the 5-HT_{1A} receptor agonist might be attributable to the effect of the drug that is not due to an action of the drug that would specifically reverse the pathophysiology leading to fatigue.[15] The aim of this study, therefore, was to study the central serotoninergic system and specifically the 5-HT_{1A} receptor–mediated pathway of serotoninergic neurotransmission using several neurochemical and biological assays.

Materials and Methods

Chemicals. [^{3}H]8-OH DPAT (211Ci/mmol) and [^{3}H]MDL 100,907 (82Ci/mmol) were purchased from Amersham Biosciences Trading GmbH (Vienna, Austria). 8-OH DPAT, 5-HT, and all other substances were purchased from Sigma Aldrich Chemie, GmbH (Taufkirchen, Germany).

Animal Model. Male Sprague-Dawley rats weighing 200 to 300 g obtained from the Başkent University Animal Laboratory (Ankara, Turkey) were used throughout the study. The animals were maintained in standard facilities with a 12-hour day/night cycle and had free access to rat chow and water. The study was approved by the Ethics Committee of the Gülhane Military Academy. All animal care was conducted in accordance with the Declaration of Helsinki.

The model of cholestasis used in our study was the well-characterized model of bile duct resection (BDR). BDR was performed during laparotomy under anesthesia induced via intraperitoneal administration of ketamine HCl (50 mg/mL) and xylazine (100 mg/mL) (v/v 8/1 [1 mL]). The bile duct was isolated, doubly ligated, and resected between ligatures as previously described.[16] Sham resection consisted of laparotomy, identification, and manipulation of the bile duct without resection or ligation. Experiments were performed on the 6th day of surgery when rats were overtly cholestatic, which was also documented biochemically (data not shown) and is consistent with other studies using this model.

Assessment of Fatigue. Fatigue was assessed using the forced swim test according to Weiss et al.[17] and Swain and Maric.[11] A swim apparatus consisting of a glass cylinder 50 cm high and 22 cm wide and filled with water (25°C) to a height of 30 cm was used. A Styrofoam cup was taped to the back of each rat, just behind the head, to prevent the rats from sinking when they ceased to move in the water. Rats were put into the swim tank for 15 minutes. In the swim tank, the rats spent the time swimming or floating. In the latter case, rats were lying on the water with all four limbs motionless. For assessment of the activity of rats in the swim tank, the time rats spent floating was quantified by two observers after administration of the 5-HT_{1A} receptor agonist 8-hydroxy(di-n-propylamine)tetralin (8-OH DPAT) at a dose of 1 mg/kg or the vehicle saline when the observers were unaware of the treatment the rats had received. Floating time was quantified for a total of 15 minutes during each assessment in three 5-minute observation periods. These experiments were performed in both sham-operated and BDR rats.

Assessment of the Serotonin Behavioral Syndrome. Five components of the serotonin behavioral syndrome—namely, flat body posture, Straub tail, head weaving, reciprocal forepaw treading, and hind limb adduction—were counted by two observers who were blinded to the treatment the rats had received. The total observation period was 15 minutes. Observation was performed in 3-minute intervals. Individual components of the serotonin behavioral syndrome were scored on a scale of severity according to Deakin and Green[18]: 0, absent; 1, just present; 2, definitely present. Thus the maximum score for the measured five components during five observations was 50. Sham-resected rats and rats with BDR were administered five different doses of 8-OH DPAT (0.25, 0.5, 1, 2, and 4 mg/kg intraperitoneally) to obtain a dose–response curve for both sham-operated and BDR rats. Behavioral assessment was started 1 minute after drug administration and was performed with an Opto Varimex 3 activity meter (Columbus Instruments, Columbus, OH). For objective assessment of the behavioral syndrome, the vertical movements of the rats were recorded during the 15-minute observation periods with the Opto Varimex 3 activity meter. This system is based on counting the number of interruptions by freely moving and standing rats on a grid of infrared beams placed 1 cm apart both horizontally and vertically.[19] Vertical movements of control rats and BDR rats were recorded at each of the five doses of 8-OH DPAT during the 15-minute observation periods. Dose–response curves for the serotonin behavioral syndrome and vertical movements of sham-operated and BDR rats were compared.

Receptor-Binding Assay. In a different set of experiments, rats with BDR and sham-operated rats where decapitated on the 6th day of surgery after anesthesia induced by intraperitoneal administration of ketamine

HCl (50 mg/mL) and xylazine (100 mg/mL) (v/v 8/1 [1 mL]). Brains were removed and dissected on a cold plate (−5°C to −10°C) according to the atlas of König and Klippel.[20] Four regions of the brain were dissected: the frontal cortex, hypothalamus, hippocampus, and striatum. Dissected brain tissues were frozen at −80°C until further use. For the receptor-binding assay, frozen brain samples were thawed and homogenized in 10 volumes of ice-cold 50 mmol/L Tris-HCl buffer (pH 7.4, including 1 mmol/L ethylenediaminetetraacetic acid, 0.2 mmol/L phenylmethylsulfonyl fluoride, 14 μg/mL aprotinin) using a glass-to-glass motor-driven homogenizer. The homogenate was centrifuged at 500*g* for 15 minutes at 4°C. The supernatant was centrifuged at 40,000*g* for 60 minutes at 4°C. The pellet obtained was washed twice via resuspension in fresh buffer and via centrifugation at 40,000*g* for 60 minutes at 4°C. The final pellet was frozen at −80°C. The protein content of the membrane preparations was estimated using the method of Lowry et al.[21] 5-HT_{1A}- and 5-HT_2-binding sites were determined by binding of [^{3}H]8-OH DPAT and [^{3}H]MDL 100,907, respectively. The membranes were incubated with 0.1 to 5 nmol/L [^{3}H]8-OH DPAT or [^{3}H]MDL 100,907 in 250 μL binding buffer for 60 minutes at 37°C, and reaction was terminated via rapid filtration using a Brandel cell harvester (Gaithersburg, MD) with Whatman GF/C filters (Middlesex, UK), followed by washing with ice-cold binding buffer. Nonspecific binding was defined as the binding of the radioligands in the presence of 0.1 μmol/L 5-HT for [^{3}H]8-OH DPAT and 1 μmol/L ketanserin for [^{3}H]MDL 100,907. Radioactivity was determined via liquid scintillation spectroscopy.

In Vivo *Brain Microdialysis.* *In vivo* brain microdialysis was performed on the 6th day of surgery for BDR and sham operation. For placement of microdialysis probes, rats were reanesthetized with intraperitoneal ketamine (100 mg/kg) and chlorpromazine (1.0 mg/kg) mixture and placed in a stereotaxic frame (Model 51600; Stoelting, Wood Dale, IL). The scalp skin was incised and the periosteum was separated from the cranium. Screws were placed for support of acrylic cement. Concentric microdialysis probes were made from 15-mm-long 24-G stainless steel tubing (Cooper's Needle Works, Birmingham, UK). Inlet and outlet tubes were made of fused silica (optical density [OD]: 0.19 mm; inside diameter [ID]: 0.075 mm) (SGE, Kiln Farm Milton Keynes, UK). The fused silica tubes were placed under a surgical microscope, and the inlet that emerges from the tip of the stainless tubing was trimmed to a length of 2 mm. A Cuprophan dialysis membrane (OD: 0.216 mm; ID: 0.2 mm) (Gambro Ltd., Huntingdon, UK) was passed over the inlet silica tubing. All the joints of the probes were sealed with epoxy resin. The microdialysis probes were covered with dental acrylic cement. The probes were implanted into the the left hypothalamic areas (coordinates: −1.8 mm anteroposterior, +1.1 mm lateral, 8.9 mm ventral to bregma) according to the Paxinos and Watson brain atlas.[22] Intracerebral perfusion samples were collected the following day.

The day after placement of microdialysis probes, rats were placed individually into Plexiglas cages. Polyethylene tubings were attached to the inlet of the microdialysis probes to collect the samples from conscious rats. Artificial cerebrospinal fluid (2.5 mmol/L KCl, 125 mol/L NaCl, 1.26 mmol/L $CaCl_2 2H_20$, 1.18 mmol/L $MgCl_2 6H_20$, and 0.2 mmol/L $NaH_2PO_4 2H_20$ [pH 7.0]) was delivered continuously via a 250-μL Hamilton syringe connected to a microinfusion pump (KD Scientific, Holliston, MA). Samples were collected at a 20-minute period during a flow rate of 0.5 μL/min of the artificial cerebrospinal fluid after an equilibration period of 1 hour. The dialysates accumulated in 0.5-mL Eppendorf tubes. The dialysates were filtrated through 0.4-μm nylon membrane filters and stored at −80°C until further use. Rats were decapitated under anesthesia as described in the Receptor-Binding Assay section, and brains were cut at 50-μm slices with a microtome. Slices were stained with thionine dye, and the exact location of probes was confirmed using the Paxinos and Watson rat brain atlas.[22]

Determination of 5-HT and 5-Hydroxyindole Acetic Acid. 5-HT and 5-hydroxyindole acetic acid (5-HIAA) were measured via high-performance liquid chromatography using electrochemical detection as previously described.[23] The chromatographic system consisted of a pump with a 100-μL loop volume (Jasco PU-980, Tokyo, Japan), a rheodyne 7725 valve, a C18 reverse phase column (15 cm and 3.9 cm in length, 4.6 mm in diameter, and 5 μm in pore size), an electrochemical detector with a glassy carbon electrode and $Ag/AgCl_2$ reference electrode (GBC, LC 1260), and a computer. The mobile phase (flow rate: 0.4 mL/min) consisted of 95% 0.5 mol/L ammonium acetate (pH 5.1) and 5% methanol. The column temperature was set at 45°C and samples were injected into the column in a 10 μL volume. Retention times of 5-HT and 5-HIAA were 12.2 and 10.8 minutes, respectively. The mobile phase was filtered through a 0.4-μm nylon membrane and degassed in an ultrasonic bath. A computer program was used for chromatographic analysis.[23]

Statistical Analysis. The unpaired Student *t* test, Mann-Whitney *U* test, and one-way ANOVA followed by Dunnet's *post hoc* test were performed where appropriate. A *P* value of less than .05 was considered significant.

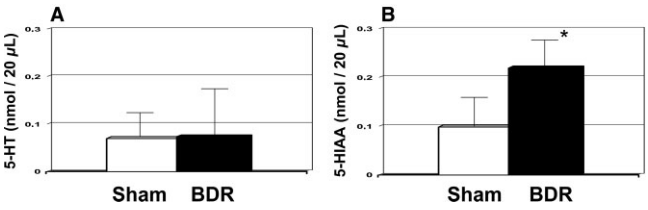

Fig. 1. Extracellular hypothalamic 5-HT and 5-HIAA levels obtained during *in vivo* brain microdialysis in sham-operated and BDR rats. (A) 5-HT levels were similar in both groups of rats; (B) 5-HIAA levels were higher in BDR rats compared with sham-operated control rats. Microdialysis probes were implanted into the the left hypothalamic areas (coordinates: −1.8 mm anteroposterior, +1.1 mm lateral, 8.9 mm ventral to bregma) according to the Paxinos and Watson brain atlas.[22] *$P = .007$. 5-HT, serotonin; 5-HIAA, 5-hydroxyindole acetic acid; Sham, sham-operated rats; BDR, bile duct–resected rats.

For assessment of dose–response curves, the nonlinear regression of 3 parameter logistic model was used.

Results

5-HT and 5-HIAA Levels During* In Vivo *Brain Microdialysis. Extracellular 5-HT levels obtained during *in vivo* brain microdialysis were similar in sham-operated rats and rats with BDR (Fig. 1A). In contrast, 5-HIAA levels were 218% higher in BDR rats compared with sham-operated control rats (Fig. 1B; $P = .007$).

Fatigue Assessment in the Forced Swim Test. Rats with BDR spent more time floating compared with sham-operated rats in the forced swim test (Fig. 2; $P < .05$). Administration of the 5-HT_{1A} receptor agonist 8-OH DPAT at a dose of 1 mg/kg had no effect in sham-operated rats but decreased the time spent floating in rats with BDL (see Fig. 2; $P < .01$). A lower dose (0.5 mg/kg) of 8-OH DPAT was without effect in the swim test in rats with BDL (see Fig. 2).

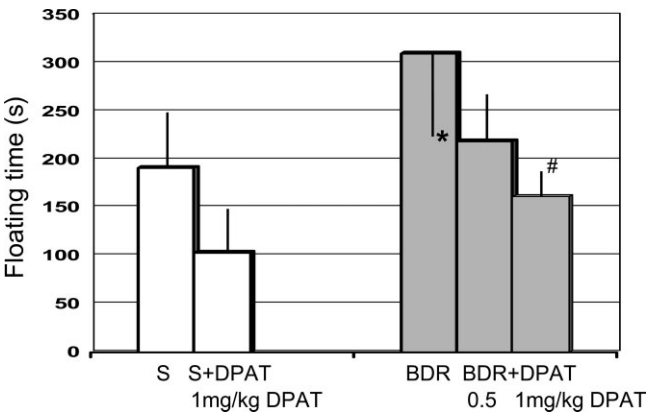

Fig. 2. Effect of the 5-HT_{1A} receptor agonist 8-OH DPAT on floating times in sham-operated rats (**white bars**) and BDR rats (**grey bars**). BDR rats spent more time floating than sham-operated rats; 1 mg/kg 8-OH DPAT led to a decrease in the time BDR rats spent floating, while 0.5 mg/kg 8-OH DPAT was without effect. 8-OH DPAT was without effect in sham-operated rats. *$P < .05$ versus sham-operated rats without treatment. #$P < .01$ versus BDR rats without treatment (n = 5 to 7 rats per group). S, sham-operated rats; DPAT, 8-hydroxy(di-n-propylamine)tetralin; BDR, bile duct–resected rats.

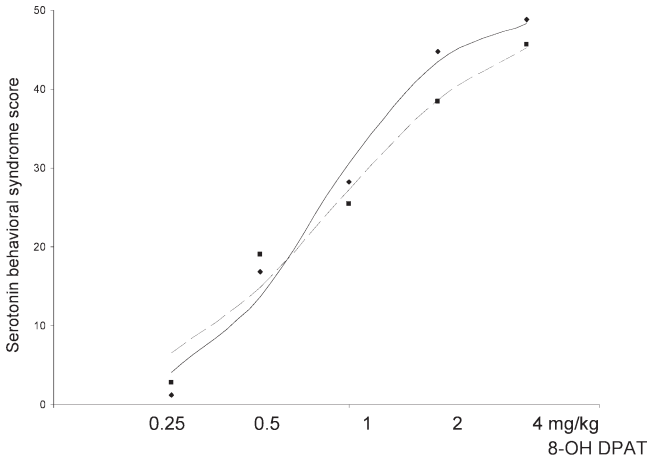

Fig. 3. The effect of escalating doses of 8-OH DPAT on the evolution of the serotonin behavioral syndrome in sham-operated (**solid line**) and BDR rats (**dotted line**). The EC_{50} of sham-operated and BDR rats was 0.89 (95% CI: 0.57-1.21) and 0.8 (95% CI: 0.59-1.02), respectively. Data are represented as the mean values of 4 to 6 determinations. 8-OH DPAT, 8-hydroxy(di-n-propylamine)tetralin.

Assessment of the Serotonin Behavioral Syndrome. The serotonin behavioral syndrome was assessed with five escalating doses of 8-OH DPAT in sham-operated and BDR rats. Components of the syndrome were not evident with the lowest dose (0.25 mg/kg), but some components started to appear in the 0.5-mg/kg dose. At the doses of 1 mg/kg and higher, the serotonin behavioral syndrome was more clearly evident. There was no difference in the dose–response curves of sham-operated and BDR rats to the escalating doses of 8-OH DPAT. Specifically, BDR rats did not appear to have an increased sensitivity to 8-OH DPAT (the EC_{50} for sham-operated and BDR rats was 0.89 [95% CI: 0.57-1.21] and 0.8 [95% CI: 0.59-1.02], respectively; the slope for sham-operated and BDR rats was 2.07 [95% CI: 1.03-3.11] and 1.5 [95% CI: 0.7-2.3], respectively) (Fig. 3). Observers were blinded to the treatment the rats received to avoid any assessment bias. Furthermore, to add an objective element to the behavioral assessment—and as a reflection of the flat body posture—vertical movements were counted. The 0.25-mg/kg dose of 8-OH DPAT did not affect vertical movements when compared with vehicle treatment; however, vertical movements decreased drastically with the 0.5-mg/kg dose, which is an indirect objective sign of the flat body posture of the serotonin behavioral syndrome. Dose–response curves for vertical movements were similar in sham-operated and BDR rats (Fig. 4). The 1-mg/kg dose of 8-OH DPAT, which substantially decreased

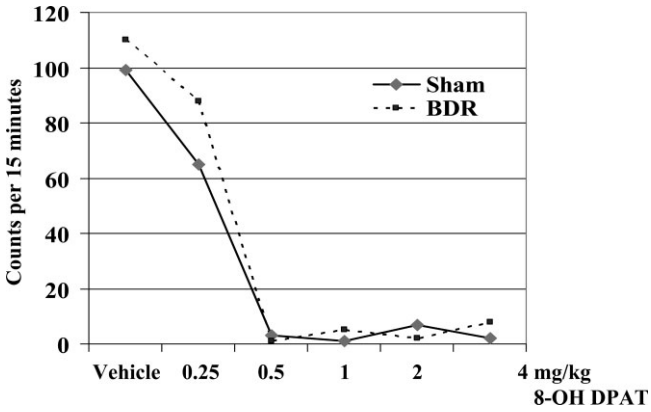

Fig. 4. The effect of escalating doses of 8-OH DPAT on vertical movements in sham-operated (**solid line**) and bile duct resected rats (**dotted line**). Data are represented as the mean values of 4 to 6 determinations. Sham, sham-operated rats; BDR, bile duct–resected rats; 8-OH DPAT, 8-hydroxy(di-n-propylamine)tetralin.

floating time in BDR rats, also completely abolished vertical movements in both sham-operated and BDR rats (see Fig. 4).

5-HT$_{1A}$ and 5-HT$_2$ Receptor Densities. The densities of binding sites for [^{3}H]8-OH DPAT (5-HT$_{1A}$ receptors) were increased in the frontal cortex in rats with BDR compared with sham-operated rats ($P < .01$) but were unchanged in the other three brain regions investigated (Table 1). The densities of binding sites for [^{3}H]MDL 100,907 (5-HT$_2$ receptors) were similar in sham-operated and BDR rats in the frontal cortex, hypothalamus, and striatum; the densities of 5-HT$_2$ receptors were increased in the hippocampus (see Table 1; $P < .05$).

Discussion

The present study consisted of two separate sets of experiments. In the first set, the status of central serotoninergic neurotransmission was investigated using *in vivo* brain microdialysis. 5-HT and 5-HIAA measurements in the hypothalamic dialysis perfusion fluid were preferred over tissue measurements, as extracellular measurements are known to reflect the functional status of the neurotransmitter more accurately avoiding the influence from other compartments, such as nerve endings and endothelial vessels.[24] Tissue measurements, on the other hand, represent a mixture of intracellular and extracellular content. The results of *in vivo* microdialysis experiments indicate that central extracellular 5-HT levels are similar in rats with BDR and sham-operated control rats, whereas levels of 5-HIAA are increased in rats with BDR compared with sham-operated control rats. These results suggest that 5-HT metabolism is increased in rats with BDR with essentially unchanged basal 5-HT output, similar to the situation in more severe liver disease with hepatic encephalopathy.[25,26] The possibility that neuronal output is actually increased but that an enhanced 5-HT uptake compensates for the change in release—thus maintaining the extracellular level at a more or less constant level—cannot be ruled out. Overall, these findings argue against a functional overactivity of the serotoninergic system as a potential cause of the fatigue associated with cholestasis. On the other hand, these data can be reconciled with decreased presynaptic 5-HT$_{1A}$ activity, because activation of 5-HT$_{1A}$ presynaptic receptors leads to decreased 5-HIAA levels. However, the inability of 8-OH DPAT at doses effective on presynaptic but not postsynaptic 5-HT$_{1A}$ receptors to ameliorate increased floating times linked to fatigue of cholestasis in the forced swim test (see later discussion) argue against a decreased presynaptic 5-HT$_{1A}$ activity as an important factor contributing to the fatigue associated with cholestasis.

In the second set of experiments, 5-HT$_{1A}$–mediated neurotransmission was investigated using biological assays and receptor-binding methodology. Previously, increased midbrain 5-HT receptor density had been reported in cholestatic rats.[27] In this area, presynaptic 5-HT$_{1A}$ receptors prevail. Thus, decreased serotonin release would be expected and consequently support the suggestion that decreased 5-HT release from nerve terminals occurs in cholestatic rats. This suggestion was further strengthened by biological assays showing enhanced hypothermic and hyperphagic responses of cholestatic rats after administration of a 5-HT$_{1A}$ receptor ligand.[27] Although these experiments provided evidence for decreased presynaptic 5-HT$_{1A}$ receptor–mediated neurotransmission in cholestasis of rats, a direct link between the fatigue of cholestasis and the potential contri-

Table 1. Equilibrium-Binding Constants for [^{3}H]8-OH DPAT and [3H]MDL 100,907 Binding to 5-HT$_{1A}$ and 5-HT$_2$ Receptors in Discrete Brain Regions From Sham-Operated and Bile Duct–Resected Rats

Treatment	Region	[^{3}H]8-OH DPAT B_{max} (fmol/mg protein)	[3H]MDL 100,907 B_{max} (fmol/mg protein)
Sham operation	Cortex	81 ± 22	139 ± 16
	Hippocampus	267 ± 87	21 ± 7
	Hypothalamus	109 ± 21	16 ± 7
	Striatum	162 ± 90	159 ± 19
Bile duct resection	Cortex	287 ± 76*	123 ± 12
	Hippocampus	207 ± 41	41 ± 5†
	Hypothalamus	158 ± 35	17 ± 5
	Striatum	171 ± 37	136 ± 16

Each value represents the mean ± SE of 3 to 6 observations, with each observation consisting of tissues harvested from 1 to 3 rats.

*$P < .01$ and †$P < .05$ versus sham-operated rats.

bution of 5-HT_{1A} receptor-mediated neurotransmission had been suggested by Swain and Maric,[11] who applied the forced swim test as a biological assay for fatigue assessment. The results of the experiments described in this article confirmed that rats with BDR spend more time floating in the forced swim test compared with sham-operated rats and that treatment with a 5-HT_{1A} receptor agonist decreases the time rats with BDR spend floating. The ameliorating effect of the 5-HT_{1A} receptor agonist 8-OH DPAT was evident with the 1-mg/kg dose. However, in studies exploring the 5-HT behavioral syndrome, 8-OH DPAT doses of 1 mg/kg and higher displayed all the components of the 5-HT behavioral syndrome. Quantitative measurement of vertical movements, as a reflection of one of the most striking components of the serotonin behavioral syndrome—the flat body posture—displayed a striking reduction of vertical movements already at the 0.5-mg/kg dose, suggesting that the serotonin behavioral syndrome started to appear already at this lower dose before its subjective detection. Furthermore, rats with BDR, compared with sham-operated rats, did not display increased sensitivity to escalating doses of 8-OH DPAT for the development of the serotonin behavioral syndrome. Finally, 5-HT_{1A} and 5-HT_2 receptor densities were unaltered in rats with BDR in most brain regions investigated when compared with sham-operated rats. Altogether, these data indicate that the 5-HT_{1A} receptor–mediated amelioration of fatigue in rats cannot be interpreted as a reversal of the pathophysiology that had led to the development of fatigue in cholestasis. Rather, the data are suggestive of a nonspecific stimulatory effect of 8-OH DPAT secondary to the intrinsic activity of the 5-HT_{1A} receptor agonist.

How can these data and their interpretation be reconciled with those of Swain and Maric?[11] In the latter experiments, rats were pretreated subcutaneously with a potent 5-HT_{1A} receptor agonist 24 hours, 5 hours, and 1 hour before they were put into a swim tank for the forced swim test. The authors claimed that this dosing regimen could result in desensitization of presynaptic 5-HT_{1A} receptors—that is, the decrease of 5-HT release from nerve terminals by 5-HT_{1A} receptor activation would fade away, and enhanced 5-HT release would ensue. It is a well-known phenomenon that repeated administration of a receptor ligand can lead to desensitization of the respective receptor; the problem, however, is whether the 5-HT1A autoreceptor was indeed desensitized in the experiments conducted by Swain and Maric. First of all, although the desensitization of 5-HT_{1A} autoreceptors has been described in several articles,[28,29] some inconsistent reports have also appeared. For example, desensitization has been reported to occur with the 5-HT_{1A} receptor agonist alnespirone, but not with 8-OH DPAT.[30] Furthermore, Swain and Maric had used LY 293284 as the 5-HT_{1A} receptor ligand in their studies, which is a much more potent 5-HT_{1A} ligand than 8-OH DPAT, the 5-HT_{1A} ligand used in the current study.[31] The dose Swain and Maric used in their studies is rather high and is expected to act on the postsynaptic receptor. A much lower dose is required for the presynaptic 5-HT_{1A} receptor, and at such a dose postsynaptic 5-HT_{1A} receptors would not be affected. Such a dose was not effective for ameliorating the increased floating time of cholestatic rats in the current study. Could it be that postsynaptic 5-HT_{1A} receptors had been desensitized? Chronic use of selective serotonin reuptake inhibitors has been reported to lead to desensitization of postsynaptic 5-HT_{1A} receptors,[32] which is not associated with downregulation of postsynaptic 5-HT_{1A} receptors but is most likely associated with mechanisms downstream from the receptor level.[33,34] In these studies, desensitization had been assessed by measuring oxytocin and adrenocorticotropic hormone responses, both representing postsynaptic 5-HT_{1A} receptor functions. We did explore the effect of the three-dose schedule (administration of 8-OH DPAT 24 and 6 hours and just before assessment) on the serotonin behavioral syndrome in the control rats. The serotonin behavioral syndrome was still evident, but the response was attenuated compared with the application as one dose (data not shown). This result suggests that even with a three-dose regimen, an attenuation but not abolishment of the single-dose effect of the ligand can be observed. Hence a "partial" desensitization most likely occurred in the experiments conducted by Swain and Maric,[11] in the frame of a decrease—but not abolishment—of the postsynaptic behavioral response.

Do the results of the present study exclude the possibility of the contribution of the serotoninergic neurotransmitter system to the genesis of fatigue associated with cholestasis, especially when the data suggestive of an unchanged central serotoninergic tone in the rat with BDR using *in vivo* brain microdialysis are considered? Several facts argue against this line of reasoning. First of all, fatigue in cholestasis has been reported in the chronic setting, whereas the experiments of this study were performed in an acute cholestasis model in the rat. Furthermore, the complexity of the serotoninergic neurotransmitter system has to be considered. At least 14 separate 5-HT receptors divided into seven main classes are recognized.[35] Thus the present study provides evidence to seriously question the role of 5-HT_{1A}–mediated neurotransmission in the genesis of fatigue associated with cholestasis. Other pathways of central serotoninergic neurotransmission were not dealt with in this study. The

case report describing relief from fatigue with a 5-HT_3 receptor antagonist in the chronic liver disease setting is therefore of interest,[36] even if the patient reported in this case report had chronic hepatitis C and not chronic cholestasis. Fatigue in cholestatic liver disease has been reported to correlate with sleep disturbance and depression,[2,37,38] both of which represent behaviors likely to be influenced by the serotoninergic system.[39] Therefore, the central serotoninergic system needs to be further investigated for its possible contribution to the genesis of fatigue associated with cholestasis.

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