Behavoural pharmacology

Effects of dopamine receptor agonist and antagonists on cholestasis-induced anxiolytic-like behaviors in rats

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Dysfunctions in the dopamine transmission system have been suggested to contribute to the pathogenesis of hepatic encephalopathy. In an experimental animal model, cholestasis induction through bile duct ligation may present several main pathological features of hepatic encephalopathy. Dopaminergic systems are shown to play pivotal roles in regulation of anxiety-like behaviors. The main bile duct in male Wistar rats, weighing 220–240 g, was ligated using two ligatures plus duct transsection in between. Anxiety-like behaviors were measured using the elevated plus maze task. Cholestasis increased the open arm time percentage (%OAT), 13 but not 10 days after bile duct ligation, indicating an anxiolytic-like effect. Sole intraperitoneal injection of apomorphine (dopamine D1/D2 receptor agonist, 0.25 mg/kg), SCH23390 (dopamine D1 receptor antagonist, 0.005, 0.01 and 0.02 mg/kg) or sulpiride (dopamine D2 receptor antagonist, 0.125, 0.25 and 0.5 mg/kg) did not alter %OAT, open arm entries percentage (%OAE) and locomotor activity in the sham-operated rats. Meanwhile, the higher dose apomorphine (0.5 mg/kg) induced anxiolytic-like behaviors in this group. The subthreshold dose injection of SCH23390 or sulpiride, partially reversed the anxiolytic-like behaviors induced by cholestasis (13 days after bile duct ligation). On the other hand, subthreshold dose of apomorphine in cholestatic rats (10 days post bile duct ligation) induced anxiolytic-like effects which could be blocked by SCH23390 or sulpiride. The effective doses of above drugs did not alter locomotor activity, number of rearings, gromings and defections. These findings suggested that the dopaminergic system may potentially be involved in the modulation of cholestasis-induced anxiolytic-like behaviors in rats.

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1. Introduction

Acute and chronic failure in liver function may give rise to cognitive and non-cognitive impairments in the brain, namely, hepatic encephalopathy (Huang et al., 2010; Magen et al., 2010; Zarrindast et al., 2012a). Cholestasis which leads to hepatic encephalopathy (Garcia-Moreno et al., 2005) is potentially associated with liver cirrhosis (Ferenci et al., 2002). The most frequently applied experimental model for induction of cholestasis is common bile duct ligation (Pi-Chieh Wang et al., 2011; Zarrindast et al., 2012a; Zhang et al., 2012). Some investigations have revealed that cholestasis decreases the anxiety-like behaviors (Eslimi et al., 2011), induces memory deficits (Cauli et al., 2009; Huang et al., 2009; Magen et al., 2010; Zarrindast et al., 2012b), tremor (Chung et al., 2005) and alters the sleep pattern (Newton, 2008). It has been made clear that cholestasis alters the activity of all classic neurotransmitter systems such as opioidergic (Roberts et al., 1987; Zhang et al., 2004) and dopaminergic (Glaser et al., 2003, 2006; Zimatkin et al., 2008).

Several studies have also indicated that the two major dopaminergic systems including mesolimbic and mesocortical are involved in anxiogenic- and anxiolytic-like responses induced by some medications or acute stress (Cabib et al., 1988; Deutch et al., 1985; Dunn, 1988; Feenstra et al., 1995; Imperato et al., 1990; Louilot et al., 1986; Nasehi et al., 2011; Puglisi-Allegra et al., 1991; Reinhard et al., 1982). Based on the biochemical and pharmacological properties, two main subfamilies of dopamine

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receptors have been described. These include dopamine D1-like (D1 and D2) and dopamine D2-like (D2, D3 and D4) subfamilies (Sealfon and Olanow, 2000).

Compelling evidence have shown that cholestasis increases the systemic opioids level (Dehpour et al., 1998; Ebrahimkhani et al., 2006; Ghafourifar et al., 1997; Talaenko et al., 2005) and this phenomenon is involved in the cholestasis-induced anxiolytic-like behaviors (Eslimi et al., 2011). Given the close link between opioidergic and dopaminergic systems with regard to anxiety-like behavior regulation (Radke et al., 2011; Rezayof et al., 2009), it is possible that opioidergic systems induce anxiolytic-like behaviors through their interactions with dopaminergic systems in different brain sites. The above interaction seems to occur within mesolimbic projections. Activation of dopaminergic neurons of nucleus accumbens and ventral tegmental area, in particular, could be mediated by the opioid peptides (Bruijnzeel, 2009; Koob and Le Moal, 2008; Radke et al., 2011).

Referring to the previous studies which have indicated dopaminergic system changes in cholestatic subjects, as well as the involvement of dopaminergic systems in regulation of anxiety-like behaviors, the aim of the present study is to find out the possible involvement of dopamine D1 and D2 receptors in cholestasis-induced anxiolytic-like behaviors in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (bred at the institute of cognitive science, Tehran, Iran), weighing 220–240 g at the time of the surgery, were used in this study. Animals were housed in Plexiglas cages in constant temperature (22 ± 2 °C) and automatically controlled light/dark cycle (12:12 h light/dark). They had access to commercial rodent pellets and water ad libitum. Four to five animals were housed in a same cage and eight were used in each experimental group. Each rat was used once only. All experimental procedures and animal use protocols were approved by the Research and Ethics Committee of the Faculty of Science, Tehran University of Medical Sciences.

2.2. Surgical procedure

Two experimental animal groups were used: sham-operated and bile duct ligation. Bile duct ligation was performed under general anesthesia induced by an intraperitoneal injection of ketamine hydrochloride 10% (50 mg/kg) plus xylazine 2% (5 mg/kg). Briefly, the common bile duct was located through a midline abdominal incision, double ligated near the liver, and transected between ligatures. Sham-operated rats underwent the same procedure except that the bile duct was manipulated without ligation or resection (to equalize the possible stress induced by surgery in both sham- and bile duct ligation operated groups). Animals were moved to their cages 5 h post operation to prevent wound dehiscence.

2.3. Elevated plus maze

Elevated plus maze is an anxiety assessment test in rodents that is used as a screening test of all currently available models of both anxiogenic and anxiolytic agents. Elevated plus maze apparatus consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 cm) with 40-cm high walls, extending from a central platform (10 × 10 cm) to make the shape of a plus sign. The whole apparatus was elevated 50 cm above the floor. The test room was illuminated by two 60-W bulbs located 1.5 m above the apparatus. Animals were placed in the junction of the four arms, after which their entries/duration in each arm as well as their other behavioral patterns (i.e., rearings, head dip) were observed for 5 min. The %OAT and %OAE, reflect the standard anxiety indices. Total arm entries were measured as an index for locomotor activity. A decrease or increase in duration of stay and entries to open arms reflect anxiogenic- and anxiolytic-like behaviors, respectively.

2.4. Other behavioral analysis

Number of rearings (the rat maintains an erect posture which is usually associated with sniffing) and groomings (the rat rubs its face, ears, mouth, vibrissae and eyes with rapid circular movements of its forepaws), as well as the defecation index (the number of boil defection) were considered as the conventional indices for anxiety-like behaviors (Casarrubea et al., 2009; Eslimi et al., 2011; Kalueff et al., 2004). Rearing, grooming and defecation data were thoroughly analyzed, although not shown here. Experiments were performed by someone blinded to the responses and statistical measurements.

2.5. Drugs

The drugs used in this study were ketamine and xylazine (Alfasan Chemical Co, Woerden, Holland), apomorphine (dopamine D1 and D2 receptor agonist), SCH23390 (dopamine D1 receptor antagonist, R(1)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-enazepine hydrochloride, Tocris, UK) and sulpiride (dopamine D2 receptor antagonist, Sigma Chemical Co., St Louis, CA, USA). All drugs were dissolved in sterile 0.9% saline. Control animals received saline or vehicle (for sulpiride). All drugs were administered intraperitoneally.

2.6. Experimental protocol

Ten and 13 days post bile duct ligation, the animals’ behaviors were tested in the elevated plus maze. Two groups of animals (i.e. sham-operated and bile duct ligation) groups were considered in each experiment.

2.6.1. Experiment 1: effects of SCH23390 on cholestasis-induced anxiolytic-like behaviors

Eight groups of animals were used in this experiment. The animals (sham-operated or cholestatic rats) received either intraperitoneal injection of saline (1 ml/kg) or different doses of SCH23390 (0.005, 0.01 and 0.02 mg/kg), 30 min prior to testing (13 days post bile duct ligation).

2.6.2. Experiment 2: effects of sulpiride on cholestasis-induced anxiolytic-like behaviors

Ten groups of animals were used in this experiment. Sham-operated or cholestatic rats received intraperitoneal injection of saline (1 ml/kg), vehicle (1 ml/kg) or different doses of sulpiride (0.125, 0.25 and 0.5 mg/kg), 30 min prior to testing (13 days post bile duct ligation).

2.6.3. Experiment 3: effects of apomorphine on cholestasis-induced changes in exploratory behaviors (10 days post bile duct ligation)

Six groups of animals were used in this experiment. Sham-operated and cholestatic rats either received intraperitoneal injections of saline (1 ml/kg) or apomorphine at doses of 0.25 and 0.5 mg/kg, 30 min prior to testing 10 days post bile duct ligation.
2.6.4. Experiment 4: effects of SCH23390 or sulpiride on anxiolytic-like behaviors induced by apomorphine in cholestatic rats

Nine groups of animals were used in this experiment. The animals received intraperitoneal injection of saline (1 ml/kg), vehicle (1 ml/kg), subthreshold doses of SCH23390 (0.005, 0.01 and 0.02 mg/kg) or sulpiride (0.125, 0.25 and 0.5 mg/kg), 30 min prior to injection of saline (1 ml/kg) or subthreshold dose of apomorphine (0.25 mg/kg) in cholestatic rats (10 days post bile duct ligation). All behaviors were assessed 30 min following the last injection.

2.7. Statistical analysis

Data were represented as mean ± S.E.M and the statistical analysis of the data was made by the analysis of variance, one and two-way ANOVA, followed by post hoc Tukey’s test. Each value displays the mean for the eight animals per group in all performed experiments. Differences with P < 0.05 between experimental groups at each point were considered statistically significant.

3. Results

3.1. Cholestasis induction

One day after bile duct ligation, the animals showed signs of cholestasis (jaundice, dark urine and steatorrhea). These signs have already been tested both qualitatively and quantitatively by other investigators (Eslimi et al., 2011; Zarrindast et al., 2012b).

3.2. Health status

Our data indicated no alteration in body weight in cholestatic rats (13 days post bile duct ligation, Mean ± S.E.M = 226.12 ± 5.7 gr) compared to the sham-operated (Mean ± S.E.M = 234.38 ± 5.8 gr) group.

3.3. Effects of SCH23390 on cholestasis-induced anxiolytic-like behaviors

Two-way ANOVA and post hoc Tukey’s analysis showed that 0.01 and 0.02 mg/kg of SCH23390, decreases the %OAT [dose effect: F (3, 56) = 5.08, P < 0.01, bile duct ligation effect: F (1, 56) = 0.23, P > 0.05, dose-bile duct ligation interaction: F (3, 56) = 5.24, P < 0.01; Fig. 1A, left and right panels], while increases the number of groomings [dose effect: F (3, 56) = 1.35, P > 0.05, bile duct ligation effect: F (1, 56) = 21.68, P < 0.001, dose-bile duct ligation interaction: F (3, 56) = 3.93, P < 0.05] and rearings [dose effect: F (3, 56) = 2.61, P > 0.05, bile duct ligation effect: F (1, 56) = 2.27, P > 0.05, dose-bile duct ligation interaction: F (3, 56) = 4.64, P < 0.01]. This however, did not alter other behavioral indices including the %OAE [dose effect: F (3, 56) = 1.06, P > 0.05, bile duct ligation effect: F (1, 56) = 0.24, P > 0.05, dose-bile duct ligation interaction: F (3, 56) = 2.65, P > 0.05; Fig. 1B, left and right panels], number of defecations [dose effect: F (3, 56) = 3.27, P < 0.05, bile duct ligation effect: F (1, 56) = 0.87, P > 0.05, dose-bile duct ligation interaction: F (3, 56) = 1.87, P > 0.05] and locomotor activity [dose effect: F (3, 56) = 1.12, P > 0.05, bile duct ligation effect: F (1, 56) = 4.92, P < 0.05, dose-bile duct ligation interaction: F (3, 56) = 1.51, P > 0.05; Fig. 1C, left and right panels] induced by cholestasis in rats (13 days post bile duct ligation).

3.4. Effects of sulpiride on cholestasis-induced anxiolytic-like behaviors

Two-way ANOVA and post hoc Tukey’s analysis showed that 0.25 and 0.5 mg/kg of sulpiride, decreases the %OAT [dose effect: F (4, 70) = 2.2, P > 0.05, bile duct ligation effect: F (1, 70) = 9.6, P < 0.01, dose-bile duct ligation interaction: F (4, 70) = 6.44, P < 0.01; Fig. 2A, left and right panels] while increases the number of rearings [dose effect: F (4, 70) = 1.34, P > 0.05, bile duct ligation effect: F (1, 70) = 4.20, P < 0.05, dose-bile duct ligation interaction: F (4, 70) = 3.29, P < 0.05]. This after all, did not alter other behavioral indices including the %OAE [dose effect: F (4, 70) = 3.46, P < 0.05, bile duct ligation effect: F (1, 70) = 6.05, P < 0.05, dose-bile duct ligation interaction: F (4, 70) = 1.15, P > 0.05; Fig. 2B, left and right panels], locomotor activity [dose effect: F (4, 70) = 0.37, P > 0.05, bile duct ligation effect: F (1, 70) = 4.31, P < 0.05, dose-bile duct ligation interaction: F (4, 70) = 0.95, P > 0.05; Fig. 2C, left and right panels], groomings [dose effect: F (4, 70) = 0.05, P > 0.05, bile duct ligation effect: F (1, 70) = 17.13, P < 0.001, dose-bile duct ligation interaction: F (4, 70) = 0.72, P > 0.05] and defecations [dose effect: F (4, 70) = 1.09, P > 0.05, bile duct ligation effect: F (1, 70) = 0.07, P > 0.05, dose-bile duct ligation interaction: F (4, 70) = 0.91, P > 0.05] in cholestatic rats, 13 days post bile duct ligation.
3.5. Effects of apomorphine on exploratory behaviors in sham-operated and cholestatic rats (10 days post bile duct ligation)

Two-way ANOVA and post-hoc Tukey’s analysis showed that, apomorphine increases the %OAT [dose effect: $F(2, 42)=17.72$, $P<0.001$, bile duct ligation effect: $F(1, 42)=8.888$, $P<0.01$, dose-bile duct ligation interaction: $F(2, 42)=1.544$, $P>0.05$; Fig. 3A, left and right panels] and %OAE [dose effect: $F(2, 42)=11.26$, $P<0.01$, bile duct ligation effect: $F(1, 42)=8.561$, $P<0.01$, dose-bile duct ligation interaction: $F(2, 42)=3.4$, $P<0.05$; Fig. 3B, left and right panels], while decreases the locomotor activity [dose effect: $F(2, 42)=10.65$, $P<0.001$, bile duct ligation effect: $F(1, 42)=0.98$, $P>0.05$, dose-bile duct ligation interaction: $F(2, 42)=2.73$, $P>0.05$; Fig. 3C, left and right panels]. This intervention however, did not alter other behavioral indices including number of rearings [dose effect: $F(2, 42)=1.67$, $P>0.05$, bile duct ligation effect: $F(1, 42)=2.23$, $P>0.05$, dose-bile duct ligation interaction: $F(2, 42)=0.23$, $P>0.05$], groomings [dose effect: $F(2, 42)=0.144$, $P>0.05$, bile duct ligation effect: $F(1, 42)=0.142$, $P>0.05$, dose-bile duct ligation interaction: $F(2, 42)=2.31$, $P>0.05$] and defecations [dose effect: $F(2, 42)=0.30$, $P>0.05$, bile duct ligation effect: $F(1, 42)=1.03$, $P>0.05$, dose-bile duct ligation interaction: $F(2, 42)=1.29$, $P>0.05$] in cholestatic rats, 10 days post bile duct ligation.

3.6. Effects of SCH23390 or sulpiride on apomorphine-induced anxiolytic-like behaviors in cholestatic rats

One-way ANOVA and post-hoc Tukey’s analysis indicated that the subthreshold dose of SCH23390 (0.01 and 0.02 mg/kg), significantly reduces the %OAT [$F(4, 35)=8.15$, $P<0.001$; Fig. 4A, left panel], locomotor activity [$F(4, 35)=4.08$, $P<0.05$; Fig. 4C, left panel] whereas, increases the number of groomings [$F(4, 35)=3.58$, $P<0.05$] and rearings [$F(4, 35)=6.97$, $P<0.001$]. This however, failed to alter %OAE [$F(4, 35)=2.4$, $P>0.05$; Fig. 4B, right panel].

Fig. 2. Effects of sulpiride on exploratory behaviors in sham-operated and bile duct ligated rats. The animals received pre-test injection of saline (1 ml/kg), vehicle (1 ml/kg) or sulpiride (0.125, 0.25 and 0.5 mg/kg) 30 min prior to testing. The exploratory behavior indices include: %OAT (A), %OAE (B) and the locomotor activity (C). Each bar represents mean ± S.E.M. **$P<0.01$ when compared to saline in the left panel and ***$P<0.01$ when compared to saline in the right panel.

Fig. 3. Effects of apomorphine on exploratory behaviors in sham-operated and bile duct ligated rats. The animals received either pre-test injection of saline (1 ml/kg) or apomorphine (0.25 and 0.5 mg/kg) 30 min prior to testing. The exploratory-like behaviors include %OAT (A), %OAE (B) and the locomotor activity (C). Each bar represents mean ± S.E.M. *$P<0.05$ when compared to saline in the left panel while **$P<0.01$ and ***$P<0.001$ when compared to saline in the right panel.
left panel] and the number of defecations \( F(4, 35) = 2.6, P > 0.05 \) induced by the subthreshold dose of apomorphine \( (0.25 \text{ mg/kg}) \) in cholestatic rats, 10 days post bile duct ligation. In conclusion, the data elicited that, SCH23390 blocks anxiolytic-like behaviors induced by apomorphine in the cholestatic rats \( (10 \text{ days post bile duct ligation}) \).

Furthermore, one-way ANOVA and post hoc Tukey’s analysis showed that the subthreshold dose of sulpiride \( (0.5 \text{ mg/kg}) \) significantly reduces the %OAT \( F(5, 42) = 4.7, P < 0.05 \); Fig. 4A, right panel], the %OAE \( F(5, 42) = 3.3, P < 0.05 \); Fig. 4B, right panel] and number of rears \( F (5, 42) = 9.87, P < 0.001 \) while increases number of gromings \( F (5, 42) = 5.54, P < 0.001 \). The above did not change other behavioral indices such as the locomotor activity \( F (5, 42) = 1.24, P > 0.05 \); Fig. 4C, right panel] and number of defecations \( F (5, 42) = 2.02, P > 0.05 \) induced by the subthreshold dose of apomorphine \( (0.25 \text{ mg/kg}) \) in cholestatic rats, 10 days post bile duct ligation. Our data suggested that sulpiride blocks the anxiolytic-like behaviors induced by apomorphine in cholestatic rats, 10 days post bile duct ligation.

4. Discussion

4.1. Effects of cholestasis on anxiolytic-like behaviors

In agreement with our previously published data, the present results demonstrated that cholestasis in rats \((13 \text{ but not 10 days post bile duct ligation}) \) induces anxiolytic-like behaviors while does not alter locomotor activity in the elevated plus maze test \((\text{Eslimi et al., 2011})\). Some studies have indicated that, acute and chronic liver failure, alter the level of activity in opioidergic \((\text{Roberts et al., 1987; Zhang et al., 2004})\), dopaminergic \((\text{Glaser et al., 2006, 2003; Zimatkin et al., 2008})\), cholinergic \((\text{Sundewall and Lefvert, 1990; Zarrindast et al., 2012a})\), GABAergic \((\text{Abboudcha et al., 2008; Ferenci et al., 1983})\), adrenergic \((\text{Borhani et al., 2005; LeSage et al., 2004})\), serotonergic \((\text{Celik et al., 2005; Nguyen et al., 2008})\) and glutamatergic \((\text{Mendez et al., 2009; Ohara et al., 2009})\) systems. Moreover, it has been documented that alterations in the release of the corticotrophin-releasing hormone \((\text{Beaumont et al., 1995; Burak et al., 2002; Swain et al., 1993})\) and the changes in brain manganese level \((\text{Forton et al., 2004})\) are involved in altered cognitive and non-cognitive behaviors induced by cholestasis. On the other hand, the mesocortical and mesolimbic dopaminergic systems’ role in anxiety-like behaviors \((\text{Nasehi et al., 2011; Talanenko et al., 2005; Zarrindast et al., 2010})\) have received much attention as the GABAergic, serotonergic and noradrenergic systems \((\text{Hayes and Schulz, 1983; Nikolaus et al., 2010; Noyes, 1985})\). Therefore, the question which raised here was whether the post cholestasis changes in the dopaminergic system activity level are involved in the anxiolytic-like behaviors induced by bile duct ligation.

4.2. Effects of dopaminergic agents on cholestasis-induced anxiolytic-like behaviors

The results indicated that, pre-test injection of apomorphine, in sham-operated rats, increases and decreases the %OAT and the %OAE, respectively. This however did not alter other behaviors suggesting an anxiolytic-like effect for the drug. On the other hand, sole pre-test injection of SCH23390 or sulpiride, at applied doses, did not alter the %OAT, %OAE and other behaviors in sham-operated rats. Moreover, pre-test administration of subthreshold doses of SCH23390 or sulpiride, decreased the %OAT however did not affect other behavioral indices induced by cholestasis \((13 \text{ days post bile duct ligation})\). The above data indicated that, both SCH23390 and sulpiride decrease the cholestasis-induced anxiolytic-like behaviors. In addition, pre-test injection of the subthreshold dose of SCH23390 or sulpiride before administration of the subthreshold dose of apomorphine in cholestatic rats \((10 \text{ days post bile duct ligation})\), decreased anxiolytic-like behaviors induced by the subthreshold dose of apomorphine in 10 days post bile duct ligation. The higher doses of SCH23390 and sulpiride however, decreased the locomotor activity and %OAE, respectively. Based on the above, the present results strongly suggest the involvement of dopaminergic system \((\text{dopamine D1 and D2 receptors})\) in cholestasis-induced anxiolytic-like behaviors. Since we did not use selective dopamine D1 or D2 receptor agonists, further experiments seem necessary to substantiate the above hypothesis. Our results are completely in line with the previous investigations showing that, both dopamine D1 and D2 receptors are involved in

![Fig. 4. Effects of SCH23390 or sulpiride on anxiolytic-like behaviors induced by apomorphine in the cholestatic rats. The animals received pre-test injection of saline (1 ml/kg), vehicle (1 ml/kg), SCH (0.005, 0.01 and 0.2 mg/kg) or sulpiride (0.125, 0.25 and 0.5 mg/kg) 15 min before saline (1 ml/kg) or apomorphine (0.25 mg/kg) injection in cholestatic rats (10 days post bile duct ligation).]
morpheine induced anxiolytic-like behaviors (Rezayof et al., 2009). It has been shown that, systemic injection of apomorphine decreases the anxiety-like effects in the elevated plus maze via the dopamine D2 receptor subtype (Garcia et al., 2005). However, our previous data indicated that, the intra-ventral hippocampal injection of apomorphine induces anxiogenic-like effects (Zarrindast et al., 2010) which may be due to the effect of apomorphine on the dopamine D2 presynaptic receptor and decrease in the dopamine level. It should be considered that in our previous experiments dopamine receptors antagonist decrease anxiogenic-like effect when were injected into nucleus accumbens (Zarrindast et al., 2012c) and ventral hippocampus (Zarrindast et al., 2010). However theses antagonists when were injected peripherally reduced anxiolytic effect by bile duct ligation. One may assume that the antagonists affected indirectly either on release or turnover of the morphine which induce anxiolytic-like activity. By the way our experiment data indicated that the antagonists have no effect on locomotor activity and this issue cannot be involved.

Moreover, some investigations have emphasized on the dopaminergic systems' pivotal role in gut functions such as intestinal motility (Anlauf et al., 2003; Li et al., 2004). This has been suggested to be via five classes of dopamine receptors which are expressed throughout the proximo-distal axis of the bowel (Li et al., 2006). The endogenous dopamine for instance, decreases the intestinal motility via dopamine D2 receptors. This indicates that, the dopamine D2 receptors are important at all levels of the bowel function (Li et al., 2006). The fact that dopaminergic system regulates the proliferation and secretion mechanism(s) of cholangiocytes in the liver, supports its role in regulation of liver function. To further elaborate on this, we may note that: 1-following cholestasis, the functional and proliferative responses of cholangiocytes are blocked by 6-hydroxidopamine (an agent inducing dopaminergic cell death) (Glaser et al., 2006); 2-ductal secretion in bile duct ligation rats are blocked by dopamine D2 receptor agonist (Glaser et al., 2003); 3-3-pressor function failure decreases the dopamine level in the frontal cortex, hippocampus (Yurdaydin et al., 1990) and striatum (Zeneroli et al., 1991). In contrast, some investigations have revealed that, hepatic encephalopathy does not alter the dopamine level in most brain regions (Bergqvist et al., 1995; Colombo et al., 1996; Hadesman et al., 1995; Wikell et al., 1998). The notion whether the change in brain dopamine level is involved in cholestasis, should be tested later.

Based on some solid evidence, the cholestasis-induced opioid tone occurs through opioid receptor stimulation by endogenous opioid peptides (Bergasa et al., 1994; Swain et al., 1992; Thornton and Losowsky, 1988). Our previous study indicated that opioidergic system (mu opioid receptor) involves in anxiolytic-like behaviors induced by cholestasis (Eslimi et al., 2011). Moreover, there are some investigations pointing out the role of dopaminergic systems in morphine induced behaviors, namely the typical anxiolytic-like effects (Eslimi et al., 2011; Radke et al., 2011; Rezayof et al., 2009), memory impairments (Zarrindast et al., 2006; Zarrindast and Rezayof, 2004) and reward (Ma et al., 2009; Narita et al., 2010). Therefore, it can be also inferred that opioids possibly exert their anxiolytic-like effects through dopamine release and dopaminergic system activation.

On the other hand, cholestasis may alter the activity of different enzymatic pathways such as the monoamine oxidase (MAO). According to literature, the hippocampal activity of this enzyme in astrocytes and radial glial cells (Mallajosyula et al., 2008) is decreased and increased two and six weeks after cholestasis, respectively (Zimatin et al., 2008). It has been also found that in thioacetamide-induced hepatic encephalopathy, the level of dopamine decreases in the frontal cortex and hippocampus (Yurdaydin et al., 1990). Moreover, in porto-systemic drug induced hepatic encephalopathy, the level of dopamine decreases in the striatum (Zeneroli et al., 1991).

The subthreshold dose of apomorphine (dopamine D1 and D2 receptor agonist) induces anxiolytic-like behaviors 10 days post bile duct ligation (which did not alter anxiety-like behaviors by itself). This response was blocked by SCH23390 and sulpiride. Furthermore, the antagonists block the cholestasis-induced anxiolytic-like behaviors in rats; 13 days post bile duct ligation. Taken all these together, we may conclude that the anxiolytic-like behaviors induced by bile duct ligation are possibly mediated through dopamine D1 and D2 receptor mechanisms.

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