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Role of nitric oxide in hypodipsia of rats with obstructive cholestasis

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Abstract

Cholestasis is associated with the overproduction of nitric oxide (NO), and NO acts as an inhibitory mechanism when thirst is stimulated by water deprivation or by angiotensin II. Due to the presence of hypodipsia in the cholestatic condition, we have compared the rate of water intake between bile duct-ligated (cholestatic) and sham-operated rats. We have evaluated the effect of NO synthesis inhibition by N^G-nitro-L-arginine (L-NNA, 10 mg kg^{-1}/day) on the rate of water intake in cholestatic rats. The results showed that plasma alkaline phosphatase activity (a marker of liver damage) increased after bile-duct ligation, and that its elevation was partially (but significantly) prevented by treatment with L-arginine. A two-week bile-duct obstruction induced a significant decrease in the rate of water intake compared with sham-operated animals (35.87 ± 1.45 vs 42.37 ± 1.99 mL/day, $P < 0.05$). This effect was corrected by the daily administration of L-NNA. Surprisingly, L-arginine (200 mg kg^{-1}/day) showed similar activity as L-NNA in cholestatic rats and increased water intake, but not in control animals. Systemic NO synthesis inhibition corrected the decrease in water intake observed in cholestatic rats. This suggests an important role for NO in the pathophysiology of hypodipsia in cholestatic subjects. The effect of chronic L-arginine administration observed in cholestatic rats but not seen in the control rats could be explained theoretically by the amelioration of cholestasis-induced liver damage by chronic L-arginine administration in bile duct-ligated rats.

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Introduction

Hypodipsia has been linked to cholestatic liver disease among other systemic, metabolic and central nervous conditions (Martinez-Rodenas et al 1989; Pereira et al 1994). Although bile duct obstruction is associated with depletion of extracellular water and hypotension, water-drinking behaviour of cholestatic subjects is attenuated (Martinez-Rodenas et al 1989) and the precise mechanism of cholestasis-induced hypodipsia is not understood completely.

Neurons capable of nitric oxide (NO) synthesis have been localized in various thirst-related hypothalamic nuclei, supporting the role of NO in the regulation of drinking behaviour (Roth & Rowland 1998). Calapai et al (1992) found that NO acted as an inhibitory mechanism when thirst was stimulated by water deprivation or by angiotensin II, and that the preoptic area might be one of the central sites of the antidipsogenic action of NO. Due to the biological evidence of NO overproduction in cholestasis (Niedergerger et al 1995; Ghafourifar et al 1997; Inan et al 1997) we have previously shown that inhibition of NO synthesis could correct

some complications of cholestasis (Dehpour et al 1998; Nahavandi et al 1999a,b; Sadr et al 1999). In this study, we have evaluated the drinking behaviour of cholestatic rats and determined whether hypodipsia of cholestatic rats could be corrected after systemic inhibition of NO synthesis.

Materials and Methods

Animal manipulation

The investigation conformed to the guide for the care and use of laboratory animals published by US National Institute of Health (NIH Publication No. 85–23, revised 1985).

Male Sprague-Dawley rats (200–250 g) were given free access to food and tap water under a 12-h light/dark cycle with constant temperature. Laparotomy was performed under general anaesthesia induced by an intraperitoneal injection of ketamine HCl (50 mg kg^{-1}) and xylazine HCl (10 mg kg^{-1}). The bile duct was isolated and double-ligated as described by Dehpour et al (1998). Sham-operated age-matched rats served as the control. Sham operation consisted of laparotomy and bile duct identification and manipulation without ligation.

Drug administration

The animals were divided into three groups, and received L-arginine (200 mg kg^{-1}/day), N^G-nitro-L-arginine (L-NNA, 10 mg kg^{-1}/day) or an equivalent volume of saline subcutaneously (Nahavandi et al 1999b). During a two-week period the volume of water intake was recorded every day. At the end of the experiment the rats were anaesthetized with sodium pentobarbital (50 mg kg^{-1}) and blood was collected for the measurement of plasma alkaline phosphatase activity (a marker of the severity of cholestasis-induced liver damage) and determination of plasma concentration of total cholesterol, using routine methods.

Drugs

L-arginine and L-NNA were purchased from Fluka, Switzerland. Ketamine HCl was purchased from Gedoon Richter Ltd, Hungary. Xylazine was purchased from Bayer AG, Germany, and sodium pentobarbital was purchased from Merck, Germany.

Statistical analysis

All data are presented as the means $\pm$ s.e.m. Statistical evaluation of the data was by analysis of variance followed by the Newman-Keuls test for multiple comparisons. A P-value less than 0.05 was considered statistically significant.

Results

One day after laparotomy, bile duct-ligated rats revealed manifestations of cholestasis (jaundice, dark urine and steatorrhea). After the animals were killed plasma alkaline phosphatase activity and total cholesterol level were significantly higher in bile duct-ligated rats compared with sham-operated rats (Table 1), which realized that bile duct-ligation induced cholestasis. Chronic L-arginine administration partially, but significantly ($P < 0.001$), prevented the elevation of alkaline phosphatase activity (Table 1).

As shown in Figure 1, bile duct-ligated/saline rats drank significantly less water than sham-operated/saline animals. This effect was corrected by the daily administration of L-NNA, and there was no significant difference between sham-operated/saline and bile duct-ligated/L-NNA animals in the volume of water intake. Surprisingly, in bile duct-ligated rats the action of L-arginine was similar to L-NNA and increased the drinking of water, but not in sham-operated animals (Figure 1).

Discussion

Obstructive cholestasis is associated with the depletion of extracellular water and the alteration of water and sodium regulating hormones (Pereira et al 1994). These alterations could explain the tendency to develop hypotension and renal failure, which are associated with obstructive jaundice (Martinez-Rodenas et al 1989). It is well known that decreases in extracellular fluid stimulate thirst as a compensatory mechanism (Fitzsimons 1998). Meanwhile in the cholestatic condition, depletion of extracellular fluid is not only accompanied by increased water-intake but is also associated with hypodipsia, which can deteriorate the prognosis of cholestasis (Pereira et al 1994). The effect of extracellular fluid depletion on thirst is mediated in part via angiotensin II (Fitzsimons 1998) and several endogenous ligands such as NO could influence angiotensin II-induced drinking (Fitzsimons 1998). Calapai et al (1992) found that NO

Table 1 Comparison of alkaline phosphatase activity (units L^{-1}) and total cholesterol level of plasma (mmol L^{-1}) in bile duct-ligated and sham-operated rats receiving L-arginine, N^G-nitro-L-arginine (L-NNA) or saline.

Group	Alkaline phosphatase activity	Total cholesterol
Bile duct-ligated/saline	310 ± 5^{a}	7.0 ± 1.5^{b}
Bile duct-ligated/L-NNA	303 ± 5^{a}	7.2 ± 1.7^{b}
Bile duct-ligated/L-arginine	$272 \pm 4^{a,c}$	7.1 ± 1.6^{b}
Sham-operated/saline	120 ± 5	1.6 ± 0.9
Sham-operated/L-NNA	115 ± 3	1.5 ± 1.2
Sham-operated/L-arginine	123 ± 4	1.5 ± 1.1

Data are shown as mean ± s.e.m. Six to eight rats were used in each group. $^{a}P < 0.001$, $^{b}P < 0.01$ compared with sham-operated groups. $^{c}P < 0.001$ compared with bile duct-ligated/saline and bile duct-ligated/L-NNA animals.

acted as an inhibitory mechanism when thirst was stimulated by water deprivation or by angiotensin II. Furthermore, it has been reported that injection of L-arginine into the lateral ventricles of water-deprived rats or rats given angiotensin II inhibited the drinking caused by these two stimuli in a dose-dependent manner (Calapai & Caputi 1996).

Vallance & Moncada (1991) proposed that NO could be responsible for hyperdynamic circulation in cirrhosis, and there has been increasing evidence that NO is implicated in the pathophysiology of liver disease such as cholestasis (Heinemann & Stauber 1995; Ghafourifar et al 1997; Inan et al 1997). Several studies have suggested overproduction of NO in cholestasis as well as in animal models of cirrhosis (Heinemann & Stauber 1995; Neidergerger et al 1995; Inan et al 1997). According to Vallance & Moncada (1991) NO overproduction may be due to increased incidence of endotoxaemia after bile duct obstruction, and endotoxaemia may induce NO overproduction directly or indirectly through cytokines. Wardle & Wright (1971) first suggested the association between endotoxaemia and cholestasis, and thereafter many studies have suggested that gut-derived endotoxins are implicated in the pathophysiology of cholestasis (Raynolds et al 1995; Inan et al 1997). For example, Inan et al (1997) showed that endotoxaemia in cholestasis may induce overproduction of NO that may lead to impairment of cyclic GMP associated vasodilatation and disrupt the autoregulation of the vascular bed. However, some studies have not supported the hypothesis of Vallance & Moncada (Fernandez et al 1995; Zimmermann et al 1996). Fernandez et al (1995) could not show any significant increase in inducible NO synthase (iNOS) activity in bile duct-ligated rats. It seemed that increase of constitutive NO synthase (cNOS) activity was responsible for NO overproduction in cholestasis (Gadano et al 1999), but the reason for this is not understood completely.

According to the biological evidence of NO overproduction in cholestasis, we have shown that inhibition of NO synthesis could correct some complications of cholestasis (Dehpour et al 1998; Nahavandi et al 1999a, b). Results from this study are in agreement with our previous work. The results showed that cholestatic rats had hypodipsia, which could be corrected with daily administration of a NO synthase inhibitor and suggested that NO might have a role in the altered drinking behaviour of the cholestatic rat. We could not show

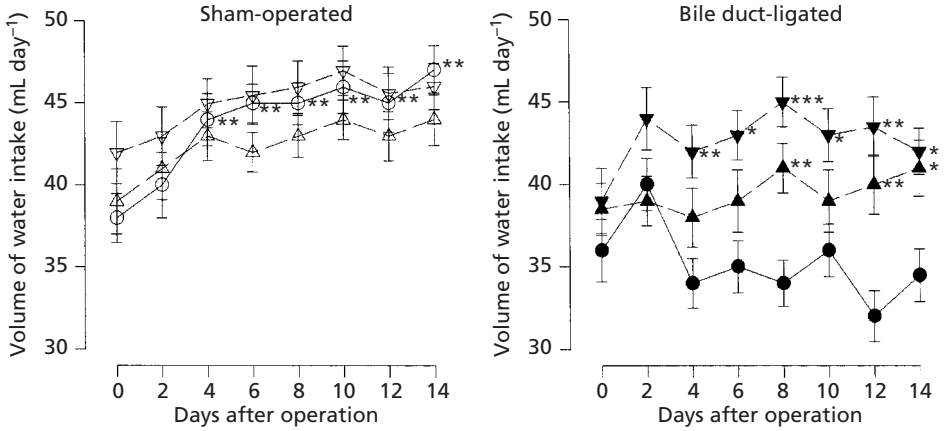

Figure 1 Comparison of the water intake between bile duct-ligated and sham-operated rats receiving saline (○; ●), L-NNA (▽; ▼) or L-arginine (△; ▲). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

significant change in the rate of water intake in sham-operated animals receiving L-NNA. The possible explanation is that NO is involved in drinking behaviour only in the volume-depleted condition (related to angiotensin II-induced water intake) while sham-operated animals were not in the hypovolaemic state. This is in agreement with Calapai et al (1992) who reported that NO synthase inhibition antagonized the effect of L-arginine in water-deprived rats but, by itself, did not increase thirst.

Surprisingly, in bile duct-ligated rats the action of L-arginine was similar to L-NNA and increased water drinking, but not in sham-operated animals. Furthermore, L-arginine treatment significantly prevented the elevation of plasma alkaline phosphatase activity, which is a marker of liver damage (Table 1). This result is in agreement with Muriel & Gonzalez (1998), who reported that cholestasis-induced liver damage in rats was ameliorated partially by L-arginine. Muriel & Gonzalez (1998) reported that bile-duct ligation doubled liver lipid peroxidation, whilst L-arginine completely prevented this change. Amelioration of liver damage could be responsible for L-arginine-induced correction of hypodipsia of cholestasis but further experiments using measurement of specific markers of liver injury as well as histopathological evaluations are necessary to prove this hypothesis.

Systemic NO synthase inhibition corrected the decreased water intake of cholestatic rats and suggested an important role for NO in the pathophysiology of hypodipsia in cholestatic subjects. The effect of chronic L-arginine administration observed in cholestatic rats but not seen in the control rats could be explained theoretically by the amelioration of cholestasis-induced liver damage by chronic L-arginine administration in bile duct-ligated rats.

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