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Seizure susceptibility alteration following reversible cholestasis in mice: Modulation by opioids and nitric oxide

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Abstract

There is an increasing body of evidence that the central nervous system is affected by cholestatic liver disorders. Cholestasis has been shown to result in a decreased seizure propensity which is believed to be mediated by an increased opioidergic tone and nitric oxide (NO) signaling pathway. In this study, we used a reversible chemically-induced cholestasis model in mice to investigate the changes in seizure susceptibility. The cholestasis was induced by intragastric administration of alpha-naphthylisothiocyanate (ANIT) (100 mg/kg) or vehicle (corn oil). The threshold to generalized clonic seizures induced by timed intravenous infusion of pentylenetetrazole (PTZ) was used as an index of seizure propensity. The role of opioid receptors and NO pathway in the changes of seizure threshold, and the responsiveness to the anticonvulsant effect of opioid agonist, morphine, during and after the resolution of cholestasis was studied in this reversible paradigm of cholestatic disease. A significant increase in cholestasis-related biochemical markers as well as in clonic seizure threshold was observed; it was maximal at day 3 after cholestasis induction and slowly decreased to normal thereafter. Seizure threshold rise was inhibited by chronic administration of the opioid antagonist naltrexone or acute administration of *N*-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO production. Co-administration of subeffective doses of L-NAME and naltrexone showed an additive effect. Injection of an anticonvulsant dose of morphine on day 7 after cholestasis induction did not increase seizure threshold, suggestive of a downregulation of receptors even after cholestasis resolution. These data shows that ANIT-induced cholestasis leads to a reversible increased resistance to PTZ-induced seizures through an opioid/NO-mediated pathway, and is probably accompanied by downregulation of opioid receptors.

Keywords: Alpha-naphthylisothiocyanate; Cholestasis; Clonic seizure threshold; Nitric oxide; Opioid; Morphine; Pentylenetetrazole

1. Introduction

Cholestasis is a systemic retention of bilirubin, bile salts, cholesterol and other solutes that are eliminated by bile. It is either caused by hepatocellular dysfunction or obstruction of intrahepatic or extrahepatic bile ducts (Sherlock and Dooley, 2002). The clinical syndrome is associated with jaundice, malabsorption, pruritus and consequently hepatic function and structure dysfunction (Goodman and Ishak, 2003; Sherlock and Dooley, 2002).

Cholestasis is accompanied by several extrahepatic manifestations including antinociception (Bergasa et al., 1994), pruritus, changes in blood brain barrier (Wahler et al., 1993), as well as changes in propensity to seizure generation (Homayoun et al., 2002b). It has recently been shown that bile duct ligation in mice is associated with a significant increase in resistance to clonic seizures which is believed to be related to elevated plasma levels of endogenous opioids and overproduction of nitric oxide (NO) (Homayoun et al., 2002b). The bile duct ligation model, although well-known and widely used model for cholestasis, is a non-reversible model which is unable to answer whether the changes in seizure propensity resolves parallel to the healing of cholestasis or whether they are long-lasting effects. Moreover, opioid receptors tend to change their responsiveness to physiologic or pharmacologic agonists. This

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may be due to desensitization or internalization of receptors (Marie et al., 2006). A key question in studying the physiologic changes attributed to the opioid system is whether those changes persist after opioids are removed. Therefore, in this study we used a reversible model of cholestasis by oral administration of α-naphthylisothiocyanate (ANIT). This drug induces an intrahepatic bile stasis which often spontaneously resolves (Chisholm et al., 1999). The seizure paradigm that we used in this study was the mouse model of myoclonic seizures induced by timed intravenous infusion of the GABA transmission blocker pentylenetetrazole (PTZ) which is very sensitive to the changes in seizure susceptibility (Mandhane et al., 2007).

Following investigation of the seizure threshold alteration during the course of this type of cholestasis, we also studied the involvement of the endogenous opioid system and NO signaling pathway. NO is a gaseous messenger molecule in a number of tissues including the brain, and has been suggested to possess both anticonvulsant (Starr and Starr, 1993; Theard et al., 1995) and proconvulsant (Nidhi et al., 1999; Osonoe et al., 1994) properties depending on the experimental conditions (e.g. animal species and type of experimental seizure). Recent studies have shown that NO is linked to opioidergic modulation of seizure threshold (Homayoun et al., 2002a; Khavandgar et al., 2002, 2003) as well as some opioidergic manifestations of cholestatic animals including opioid tolerance, analgesia and naloxone-precipitated withdrawal signs (Dehpour et al., 1998; Ghafourifar et al., 1997). In order to test the hypothesis that the exaggerated opioid response in the brain during the course of cholestasis might lead to more persistent effects, we also challenged the responsiveness of the animals to the anticonvulsant effect of an opioid agonist, morphine (with doses as low as 1–3 mg/kg) (Homayoun et al., 2002a; Honar et al., 2004; Riazi et al., 2004) after obstruction resolution.

2. Materials and methods

2.1. Drugs

Pentylenetetrazole (PTZ), naltrexone and *N*-nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma (Pool, UK). α-Naphthylisothiocyanate (ANIT) was purchased from Sigma (Toronto, Canada). Morphine sulphate was a generous gift from Temad (Tehran, Iran). ANIT was dissolved in corn oil (100 mg/ml) and naltrexone and L-NAME were freshly made in sterile physiological saline. All injections were done with the volume of 10 ml/kg of the bodyweight of the mice. PTZ was prepared in physiological saline as a 1% solution.

2.2. Animals

Male Swiss mice (Razi Institute, Karaj, Iran), weighing 26–30 g were used. The animals were housed at a constant room temperature (~25 °C) and exposed to a 12 h light, 12 h dark cycle with free access to food and water. They were housed in standard polycarbonate cages and acclimated at least 2 days before experiments. The experiments were always conducted between 9:00 a.m. and 3:00 p.m. Each group in the study consisted of 6–8 animals. The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by National Institutes of Health (NIH publication no. 85-23; revised 1985) and with the recommendations and approval of the Ethics Committee on Animal Experiments of the institution (Medical Sciences/University of Tehran). Each animal was tested only once and was immediately euthanized after seizure threshold test or blood sampling.

2.3. Chemical cholestasis model

The mice underwent fasting for 8–12 h before ANIT administration. ANIT was delivered by intragastric (IG) gavage with a concentration of 100 mg/kg (Chisholm et al., 1999). Sham animals only received an IG dose of corn oil with the same volume.

2.4. Biochemical measurements

Immediately after euthanasia, blood samples were taken via cardiac puncture (about 0.5 ml/mouse). Kits for plasma bilirubin (total) and alkaline phosphatase measurement (ZiestChem Diagnostics, Tehran, Iran) were used and the data was extracted using spectrophotometry according to the provided protocol. Sufficient amounts of plasma for bilirubin and alkaline phosphatase assays were 50 µl and 20 µl, and the sensitivity of assays were declared 0.1 mg/dl and 5 IU/l by the manufacturer, respectively.

2.5. PTZ-induced seizure threshold

The threshold of PTZ-induced seizures was measured by an infusion of PTZ solution into the tail vein of freely moving mice at a constant rate of 0.6 ml/min via a 30-gauge needle, connected by a polyethylene tube to a Hamilton microsyringe (Loscher et al., 1991). Minimal dose of PTZ (mg/kg) required to induce general clonus was recorded. General clonus was characterized by forelimb clonus followed by whole body clonus.

2.6. Experiments

To determine the time course of the changes of clonic seizure threshold after ANIT-induced cholestasis, we measured the clonic seizure threshold on the ANIT administration day, and the next 14 consecutive days in comparison with sham group (mice gavaged with plain corn oil). Biochemical markers (plasma levels of bilirubin and alkaline phosphatase) were also measured daily to determine the status of cholestasis in mice in the days following ANIT treatment.

We also examined the modulatory effects of opioids and NO on clonic seizure threshold in chemically-induced cholestasis using naltrexone and L-NAME. Groups of ANIT-treated, sham (corn oil-treated), and untreated control animals received chronic daily intraperitoneal (IP) injections of naltrexone (0 (saline), 2, 5 or 10 mg/kg) with the first injection 2 h before ANIT administration. Seizure thresholds were examined 3 days after ANIT administration which represented the day of the