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Effects of aspirin on immobile behavior and endocrine and immune changes in the forced swimming test: Comparison to fluoxetine and imipramine

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ABSTRACT

Aspirin (ASP) is the most commonly used non-steroidal anti-inflammatory drug in the world. Recent clinical and preclinical evidence suggests that ASP may also exert psychoactive effects. It remains unclear whether ASP has antidepressant-like activity, and any molecular mechanisms underlying such activity have yet to be elucidated. Using the forced swimming test (FST), a well-established animal model of depression widely used to screen potential antidepressants in rodents, we investigated the effects of subacute treatment with ASP (0, 6, 12, 25, and 50 mg/kg, i.p.) on immobility in the FST, and on FST-induced changes in endocrine and immune parameters in rats, in comparison to the clinical antidepressants imipramine (IMI) and fluoxetine (FLU). Serum levels of corticosterone, pro-inflammatory cytokine interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) were determined by enzyme-linked immunosorbent assay. ASP dose-dependently decreased immobility in the FST, without altering the locomotor activity in the open-field test. The inhibitory effects of higher doses (25 and 50 mg/kg) of ASP on immobility were similar to that of FLU and IMI at a dose of 10 mg/kg. In addition, the levels of corticosterone, IL-6, and TNF-α in peripheral blood were significantly increased after the FST exposure. IMI, but not FLU and ASP at any dose tested, significantly attenuated corticosterone responses in the FST. Both FLU and IMI treatment reduced the increase of IL-6 and TNF-α levels following the FST exposure. ASP dose-dependently decreased FST-induced increase of cytokine levels, as manifested by significantly stronger effects on IL-6 and TNF-α levels at higher doses (25 and 50 mg/kg) than the lowest dose of ASP (6 mg/kg). In all, these results indicate that ASP treatment dose-dependently decreased the immobility time and the release of pro-inflammatory cytokines in the FST, suggesting that the anti-inflammatory effects of ASP might be involved in the antidepressant-like effect.

1. Introduction

Aspirin (ASP, acetylsalicylic acid) is perhaps the most commonly used non-steroidal anti-inflammatory drug (NSAID) in the world. It is widely accepted that ASP has anti-inflammatory, analgesic and anti-pyrexic effects, and prevents the aggregation of platelets (Ferreira et al., 1973; Roth and Majerus, 1975; Vane and Botting, 2003). In recent years, some clinical evidence suggests that ASP may also possess mood-modulating effects. For example, regular ASP use as a prophylactic therapy for ischemic heart disease was associated with lower anxiety and depressive symptom scores (Ketterer et al., 1996; Sarkar et al., 2011) and a lower risk for major depressive disorder (MDD) (Charlton, 2009; Pasco et al., 2010). When given in combination with the selective serotonin reuptake inhibitor (SSRI) fluoxetine (FLU), ASP accelerates the onset of action in depressive humans and rats (Brunello et al., 2006; Mendlewicz et al., 2006) and can serve as an effective adjunct to FLU treatment in treatment resistant depressive rats (Mendlewicz et al., 2006; Wang et al., 2011). But inconsistent with these studies, Warner-Schmidt and colleagues reported that the effects of SSRIs were attenuated by anti-inflammatory drugs (ibuprofen and acetylsalicylic acid) both in mice and humans (Warner-Schmidt et al., 2011). Until now, it remains unclear whether ASP has direct antidepressant-like activity.

The forced swim test (FST) is a behavioral paradigm that has been considered as a model of the depressive state and is widely used as a predictor of antidepressant activity in rodents (Cryan et al., 2002; Lucki, 1997; Porsolt et al., 1977). When animals are exposed to the

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FST they typically display an immobile posture that is considered to reflect a state of "behavioral despair" on the assumption that the animals have given up hope of escaping (Porsolt et al., 1977). Therefore, exposure to this swim stress paradigm produces a change in behavior that is thought to model a kind of depression-like phenotype, namely despair or helplessness (Borsini and Meli, 1988; Cryan et al., 2002; Detke et al., 1995; Porsolt et al., 1977). It is well established that subacute treatment with most classes of antidepressants including SSRIs, selective noradrenaline reuptake inhibitors (SNRI), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs) reduces the duration of immobility in the FST (Borsini and Meli, 1988; Cryan et al., 2005; Kelliher et al., 2003; Kulkarni and Dhir, 2007; Lucki, 1997). In the present study, we investigated whether ASP exerts antidepressant-like effects, as indexed by reduced immobility durations in the FST.

In addition to being a useful screening test for antidepressant activity, the FST is also a potent psychophysiological stressor that causes a series of endocrine and immune function changes similar to that reported in depressed patients and animals (Connor et al., 1997, 1998). Firstly, depression is associated with hypercortisolemia (Young et al., 2004). It has been shown that FST exposure induced the increase in serum corticosterone concentration (Connor et al., 1997; Duncan et al., 1998), and some classes of antidepressants, such as TCAs and MAOIs, can attenuate this change (Connor et al., 2000; Flugy et al., 1992; Reul et al., 1993, 1994). Pharmaceutical and transgenic studies further demonstrate that animals with hyper-reactive or hypo-reactive HPA axes exhibit higher or lower immobility in the FST (Johnson et al., 2006; Jutkiewicz et al., 2005; Tronche et al., 1999), suggesting that HPA axis activities modulate immobility in the FST. ASP has been reported to have anti-stress effects. For example, ASP pretreatment reduces the cortisol awakening response (Watson et al., 2009) and inhibits the adrenocorticotropic hormone (ACTH) and cortisol responses to arginine vasopressin (AVP) (Nye et al., 1997). Thus, one potential way that ASP may modulate immobility in the FST is to regulate HPA axis responses. Secondly, hyperactivities of pro-inflammatory cytokines have been consistently observed in human beings and animals with depressive symptoms (Maes, 1995, 1999; Yirmiya et al., 2000), such as an increase in circulating levels of IL-6 (Frommberger et al., 1997; Motivala et al., 2005; Pike and Irwin, 2006; Zorrilla et al., 2001) and TNF-α (Anisman et al., 1999; Yirmiya et al., 2000). FST can induce significant increases in IL-6 and TNF-α level in rodents, which can be ameliorated by various antidepressant treatments (Castanon et al., 2002; Xia et al., 1996). Also, mice with the IL-6 or TNF-α genes knocked out or over-expressed exhibit decreased or increased immobility in the FST, respectively (Chourbaji et al., 2006; Kaster et al., 2012; Wu and Lin, 2008; Yamada et al., 2000). This suggests that proinflammatory activities modulate immobility in the FST. As an anti-inflammatory drug, it is well known that ASP suppresses various inflammatory reaction processes (Amann and Peskar, 2002). For example, ASP treatment suppresses the levels of many kinds of pro-inflammatory cytokines, including TNF-α and IL-6 (De Cristobal et al., 2002; Gao et al., 2009; Ikonomidis et al., 1999; Joussen et al., 2002; Mastbergen et al., 2002; von Känel et al., 2008). Therefore, another potential way that ASP modulates immobility is to regulate immune changes induced by FST exposure.

The present study was designed to investigate the effects of subacute ASP treatment on immobility, endocrine and immune alterations in the rat FST paradigm, in comparison to two kinds of clinically used antidepressants, the SSRI FLU and the TCA imipramine (IMI), in the same task.

2. Materials and methods

2.1. Animals and drug treatment

Male Sprague–Dawley rats, weighing 220–250 g, were obtained from the animal center of the Academy of Chinese Military Medical Science (Beijing, China). Experiments were not initiated until at least 1 week after they arrived in the colony to allow for adaption to the laboratory environment. All rats were singly housed under a 12 h/12 h light/dark cycle with lights on at 07:00 in a temperature-controlled room (22 ± 1 °C). Food and water were available ad lib at all times. Animals were briefly handled for 3 min once a day for 4 days before the beginning of the experiment to make them acclimate to the handling during the experimental procedures. The following drugs were used: sodium salt of acetylsalicylic acid (Sigma-Aldrich Co., Louis., MO, USA), IMI hydrochloride (Sigma-Aldrich), and FLU hydrochloride (Sigma-Aldrich). All drugs were diluted in saline (0.9% w/v NaCl) and intraperitoneally injected in a volume of 1 ml/kg. Rats were randomly divided into two groups based on forced swim exposure: FST (n = 49) and control (CON) (n = 42). Each group included seven subgroups: the saline (SAL) group, IMI group (10 mg/kg), FLU group (10 mg/kg), and aspirin (ASP) groups at the doses of 6, 12, 25, and 50 mg/kg. All drug injections were given three times within 24 h (23.5, 5 and 1 h prior to the second FST exposure). The ASP dose range of 6–50 mg/kg was chosen because 50 mg/kg is dose with clinical efficacy without obvious side effects (Kelton et al., 1978; Liu et al., 2003; Michel et al., 2003; Wang et al., 2011). The dose values For FLU (10 mg/kg) and IMI (10 mg/kg) were chosen as they produced the robust effects in the FST in previous studies (Reneric et al., 2002).

The experimental protocol was approved by the National Institutes of Health Guide for Care and Use of Laboratory Animals Research, as well as the Institutional Review Board of the Institute of Psychology, the Chinese Academy of Sciences.

2.2. Behavioral tests

2.2.1. Open field test

Some drugs that are not effective clinically as antidepressants, such as psychostimulants, can also decrease immobility in the FST by motor activating effects (Cryan et al., 2005). Because of this pattern, the open field (OF) test is commonly used in combination with the FST to eliminate nonspecific effects of antidepressant treatments (Gutiérrez-García and Contreras, 2009; Kaster et al., 2012). To confirm that effects of ASP on immobility were not secondary to a nonspecific increase in locomotor activity due to the drug treatment, distance traveled in an OF after drug treatment was tested 30 min before the second FST session in the present study. The testing apparatus was a circular arena of 180 cm in diameter, with a 50 cm high wall. The test room was dimly illuminated (40 LM) to decrease the averseness to the test. One rat was placed in the center of the field, and its horizontal activity (distance traveled) was recorded for 5 min and analyzed by a computer-based system (EthoVision, Noldus Information Technology, Netherlands). The open field was cleaned after each test (Qi et al., 2006; Shao et al., 2009).

2.2.2. FST

This paradigm was performed in a similar manner to that described elsewhere (Lucki, 1997). On the first day of the swim stress procedure, rats were forced to swim in a glass cylinder (50 cm height, 25 cm diameter) containing no less than 30 cm of water maintained at 23–24 °C. Rats were left to swim for 15 min before being removed. After that, rats were dried and then transported back to their home cages. The animals received a saline/drug injection 30 min after the first FST exposure. Control rats remained in their home cages at all times, and received their saline/drug injections at an equivalent timepoint. The final two saline/drug injections were administered 5 h and 1 h prior to the second FST exposure 24 h later. In the second FST exposure rats were allowed to swim for a duration of 5 min. The 5 min test session was videotaped from above using a Sony Camcorder. Behavioral analysis proceeded as described elsewhere (Cryan et al., 2002; Detke et al., 1995). Briefly, the immobility time of each rat was measured with a stopwatch by a trained observer who was blind to the experimental treatments.