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Toxicological aspects of the interesterified-fat from processed foods: Influences on opioid system and its reward effects in rats

Laura H. Milanesi[a], Karine Roversi[a], Caren T.D. Antoniazzi[a], Hecson J. Segat[b], Maikel Kronbauer[a], Lívia F. D'avila[a], Verônica T. Dias[a], Marcel H.M. Sari[b], Raquel C.S. Barcelos[a], Luana H. Maurer[c], Tatiana Emanuelli[a,c], Marilise E. Burger[a,b,c,*], Fabíola Trevizol[a,**]

[a] Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria (UFSM), RS, Brazil
[b] Programa de Pós-Graduação em Bioquímica Toxicológica, UFSM, RS, Brazil
[c] Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos, UFSM, RS, Brazil

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ABSTRACT

Considering the high consumption of processed foods, interesterified fat (IF) has been used to replace *trans* fat, since it may harm nervous system functions. Opioids are intensely used to alleviate pain, and have a highly addictive potential. Therefore, their improper use is related to addiction, tolerance, and withdrawal syndrome. *Wistar* rats received soybean oil (SO) or IF during gestation, lactation and post-weaning until pups' adolescence. On post-natal day 39, animals received morphine (4 mg/kg i.p.) in the conditioned place preference (CPP) paradigm. SO group showed morphine preference during drug withdrawal, while IF group showed no preference or withdrawal symptoms, but higher sensitivity to thermal stimuli than SO group. Morphine contidioning increased dopamine 1 receptor (D1R) and NMDAR: N-methyl-D-aspartate receptor (NMDAR) immunoreactivity in the hippocampus of SO, whereas these molecular changes were not observed in IF group. Regardless of morphine conditioning, IF group showed increased Kappa opioid receptor (KOR) immunoreactivity in the spinal cord, evidencing a negative correlation with thermal sensitivity. The chronic consumption of IF-rich foods during earlier periods of life may affect opioid neurotransmission, resulting in loss of rewarding effects related to this system.

1. Introduction

It has already been described in the literature that lifestyle, especially eating habits, can exert significant influences on reward pathways that are related to development and maintenance of addiction (Carter et al., 2016). In this sense, our research group has unstintingly been working to show that *trans* fatty acids (TFA) from enriched diets can be incorporated into neural membranes, increasing risks for neuropsychiatric conditions (Trevizol et al., 2014; Pase et al., 2013, 2015), thus modifying addiction parameters (Kuhn et al., 2015; Roversi et al., 2016) that are possibly related to changes in dopamine neurotransmission (Acar et al., 2003). TFA consumption has been associated with harmful effects to health, and in view of that, it is not recommended during pregnancy and lactation (Magri et al., 2015). Also, maternal nutrition during pregnancy and lactation determines a significant transfer of essential fatty acids through the placenta and milk to the fetus and may interfere with the development of the fetal nervous system (Souza et al., 2012). Neuringer et al. (1988) have pointed out that the greatest absorption of fatty acids (FA) by brain membranes occurs mainly during the initial stages of life. This absorption is related to affecting the neural membrane phospholipids composition and influencing their structure and neurotransmission (Fernstrom, 1999).

TFA have been reduced or eliminated from processed foods through the use of technologies such as interesterification of fats (Farfán et al., 2013). This process involves FA randomization through re-arrangement within and between triacylglycerol molecules, by either enzymatic or chemical methods, in order to obtain a good yield of desirable melting characteristics (Robinson et al., 2009). Interesterification results in improvements in physical and chemical characteristics which are

* Corresponding author.Pharmacology Post Graduation Program, Universidade Federal de Santa Maria (UFSM), Brazil.
** Corresponding author.
E-mail addresses: laura-milanesi@hotmail.com (L.H. Milanesi), karineroversi_@hotmail.com (K. Roversi), carenantoniazzi@yahoo.com.br (C.T.D. Antoniazzi), hecson_segat@hotmail.com (H.J. Segat), maikel_kr@hotmail.com (M. Kronbauer), liviadavila@hotmail.com (L.F. D'avila), vel_td@hotmail.com (V.T. Dias), marcelsarih@hotmail.com (M.H.M. Sari), raquel.barcelos@hotmail.com (R.C.S. Barcelos), luanahmaurer@gmail.com (L.H. Maurer), tatiana.emanuelli@ufsm.br (T. Emanuelli), marilise.burger@ufsm.br (M.E. Burger), fatrevizol@yahoo.com.br (F. Trevizol).

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important for the food industry (Farfán et al., 2013). Some authors have shown that the interesterification process may increase metabolic risks involved in type-2 diabetes, cardiovascular disease (Robinson et al., 2009), and atherosclerosis (Afonso et al., 2016). Considering the recent introduction of IF in the manufacturing of processed foods, there are few studies on the consequences of their consumption for health during early development, especially in the central nervous system (CNS) functions. In this sense, the CNS is the pivotal target of opioids such as morphine, which is an effective analgesic to relieve different types of pain in clinical use. However, over the years, their use without therapeutic purposes has increased (Manchikanti et al., 2012). In the last 10 years, the number of opioid prescriptions has increased by 48% in the U.S. (Manchikanti et al., 2012; Fischer et al., 2014), while its non-medical use affected 4.5 million individuals in 2013 (SAMHSA, 2014). Their prolonged use is associated with development of tolerance and hyperalgesia, in addition to physical and psychological dependence, which are related to withdrawal syndrome (Hutchinson et al., 2011). In fact, opioids have a highly addictive power and lead the addict to lose control over their intake, what results in social and health consequences (Milton and Everitt, 2012). Anxiety, hypersensitivity to pain, and drug-seeking (Zhang et al., 2016) are characteristics of opioid withdrawal, which may be associated with negative emotional states and contributes to relapse after abstinence periods (Koob and Le Moal, 2005). Opioid drugs exert their therapeutic action by binding to G protein-coupled opioid receptors such as mu (MOR), delta (DOR), and kappa (KOR). While MOR have been extensively studied in relation to opioids (Garzón et al., 2012; Ozaki et al., 2003), literature is still deficient in studies involving morphine addiction and KOR, which are involved in the loss of reward effects, since its activation reduces the release of DA (Margolis et al., 2003; Chefer et al., 2005). In fact, activation of the opioid system is related to changes in dopamine (DA) and glutamate signaling, which are responsible for the development of addiction (Sikora et al., 2016).

Considering critical periods of life such as gestation and lactation (Pase et al., 2013), and the current Western dietary habits that include chronic consumption of interesterified fats from processed foods, here we assessed the influence of chronic consumption of these fats on morphine addictive properties in adolescent pups, which are more susceptible to the rewarding effects of drugs (Roversi et al., 2016). For this purpose, behavioral tests related to morphine preference and withdrawal, as well as neurochemical changes involving dopamine, glutamate and opioid system mediated signaling through the Dopamine 1 receptor (D1R), N-methyl-D-aspartate receptor (NMDAR) and kappa opioid receptor (KOR), respectively, were assessed.

2. Experimental procedures

2.1. Animals and experimental procedure

Twelve pregnant *Wistar* rats from the breeding facility of Universidade Federal de Santa Maria (UFSM), RS, Brazil, were individually kept in Plexiglas cages with free access to food and water in a room with controlled temperature (22 ± 2 °C) and on a 12 h-light/dark cycle with lights on at 7:00 a.m. This study was approved by the Animal Ethics Committee of Universidade Federal de Santa Maria (041235/2016-UFSM), affiliated to the Council for the Control of Animal Experiments (CONCEA), following international norms of animal care and maintenance, as described in ARRIVE Guidelines (Animal Research: Reporting of In vivo Experiments) (Kilkenny et al., 2011).

Pregnant *Wistar* rats were randomly distributed into two experimental groups (n = 12) according to oral supplementation: Soybean oil (SO, rich in n-6 FA; Camera®, Santa Rosa-RS, Brazil), used as control group considering its high consumption worldwide (Zhang et al., 2012); and interesterified fat (IF, Triângulo Alimentos LTDA, Itópolis-SP, Brazil). The profile of fatty acids present in each supplementation was determined by gas chromatography (Hartman and Lago, 1973) (Table 1).

Table 1
Fatty acid composition (% of total identified FA) of the chow and the different dietary supplementation.

Fatty acids	Chow	SO	IF
Σ SFA	25.50	18.00	55.30
Σ MUFA	34.40	26.00	33.70
Σ n-6	37.30	50.30	10.10
n-3	2.90	5.50	0.30
n-6/n-3	13.00	9.20	31.6

SFA: saturated fatty acids; MUFA: Monounsaturated fatty acids.
SO: soybean oil; IF: interesterified fat.

Animals were orally supplemented (3 g/kg, p.o. once a day) (Kuhn et al., 2015; Pase et al., 2013) during gestation and lactation. After weaning, female pups of each litter were maintained in the same maternal supplementation until adolescence, on postnatal day (PND) 38.On PND 39, animals were assigned into four experimental groups, considering their previous supplementation: SO-vehicle, SO-morphine, IF-vehicle, IF-morphine; (n = 6 each group), and were exposed to the behavioral assessments as described below (Fig. 1).

Our experimental protocol was performed with female pups following previous reports that show female rats are more susceptible to imbalance in a fat diet and to drug reward addictive effects such as opioids (Jen et al., 2009; Roversi et al., 2016).

2.2. Drugs and solutions

Morphine sulfate (São Paulo, Brazil) was diluted in 0.9% NaCl solution and injected intraperitoneally (i.p.) in a dose of 4 mg/kg (Vey et al., 2015; Roversi et al., 2016). Vehicle injections were 0.9% NaCl solution.

2.3. Conditioned place preference (CPP) procedure

The CPP procedureis a well-established behavioral paradigm that has been widely employed to assess symptoms of craving, reward, extinction and relapse to addictive drugs (Vey et al., 2015). It uses a three-compartment box separated by manual guillotine doors: two compartments of equal size (45 cm × 45 cm × 50 cm) with equivalent light intensity but different visual clues: one with white floor and striped walls, and other with striped floor and smooth white walls. These two compartments converge to a third smaller compartment. The apparatus was cleaned with alcohol 20% using wet sponge and paper towel before the introduction of each animal. CPP methods have been previously described in detail (Vey et al., 2015) and were performed through the following steps: habituation, pre-test, conditioning and test. On day 1, rats (PND 39) were kept for 15 min in each compartment for habituation. The aim of this procedure is to eliminate exploratory behavior, which is common in new environments, for both pre-testing and conditioning phases, thereby avoiding misinterpretations. On day 2 (PND 40), we performed the pre-test, which consists of letting the animal freely choose one of the compartments for 15 min. Animals that showed preference (more than 70%) for any compartment were excluded from the experimental protocol. On the following 4 days, animals were conditioned with morphine (4 mg/kg i.p.) for 45 min in the compartment they spent less time during the pre-test, and with vehicle in the paired compartment, with an interval of 4 h between each conditioning. The control group was injected with vehicle (0.9%NaCl) on both sides of the apparatus. On the 5th experimental day (testing day), rats were individually placed at the center choice chamber with free access to both compartments for 15 min. Time spent in the drug-paired environment was interpreted as morphine preference.