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EICOSANOIDS IN OPIATE-INDUCED PHYSICAL DEPENDENCE IN RATS

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Abstract. Prostaglandins and leukotrienes are important autacoids of the central nervous system that intervene in normal and pathologic processes. These eicosanoids could play important roles in morphine-induced withdrawal syndrome, as proven in different experimental setups. In our studies, synthetic analogues of PGF2alfa (cloprostenol) and PGE1 (misoprostol) and a leukotriene LT1 receptor antagonist (montelukast) have influenced some of the symptoms of morphine (M) withdrawal (without interfering with other symptoms) in rats. Cloprostenol has significantly influenced certain symptoms: grooming, aggressive postures, teeth chattering, compulsive mastication, and explorations (e.g. grooming 17±2.5 in M+ClPG group vs 37±4.5 in M group) but not the others. Misoprostol (Mis) also has a differentiated effect, reducing: compulsive mastication, jumpings, aggressive positions and penile erection [e.g jumpings from 8.7±0.45 in morphine (M) group to 1.3±0.066 in M +Mis 100 ($p<0.01$); 4±0.2 in M+Mis 50 ($p<0.01$) and 7.6±0.4 in M+Mis 5 (NS)]. Our data plead for a selective and differentiated influence of PGE1, PGF2α and peptidoleukotrienes in opiate-induced physical dependence.
Keywords: prostaglandin, leukotriene, opiate, physical dependence, montelukast

Eicosanoids are biologically active lipids with 20-carbon essential fatty acids, one of the most representative systems of local hormones. They are synthesized in all cells of the human body and have many roles. Several pathways are known for metabolizing arachidonic acid: cyclooxygenase pathway, lipooxygenase pathway, monooxygenase – cytochrome P450 dependent and a non enzymatic pathway. The most important pathways of eicosanoid metabolism are the cyclooxygenases (COX1 and COX2) pathway – that induces synthesis of prostaglandins, prostacycline and thromboxanes and the lipoxygenases (LOX) pathway of particular interest being 5-LOX that induces synthesis of leukotrienes. In the brain, eicosanoids are synthesized by neurons, glial cells, endothelial and blood cells. The main group of eicosanoids synthesized in neurons and glial cells are prostaglandins. In the brain, PGE_2, PGD_2 $PGF_{2\alpha}$ and PGI_2 but also leukotrienes (LTC_4, LTB_4,) and other eicosanoids [25] are synthesized. Some of the most important functions of eicosanoids related to the brain are modulation of signal transduction, release of neurotransmitters and function of neuronal receptors.

Addiction is defined as a "compulsive behavior, reinforcing behavior (rewarding or pleasurable), loss of control in limiting intake, habitual use with an uncontrollable craving for drug" and it includes psychical and physical dependence. Chronic use of most of the drugs (with the exception of hallucinogens) induces tolerance and in conditions of abrupt cutting of drug intake a withdrawal syndrome

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occurs, representing physical dependence. Some of the most studied abuse drugs are morphine and its semisynthetic analogue, heroine. Many studies have searched for the neural substrate involved in the withdrawal syndrome. So far, there are evidences for the implication of diverse brain regions in the expression of the signs of morphine withdrawal, such as the locus coeruleus [1,12], the periaqueductal gray [3], the ventral tegmental area [2], the amygdala [4,11], the nucleus accumbens [9], several thalamic or hypothalamic nuclei [23] the spinal cord (figure 1). All the above structures are involved in different degrees in morphine-induced physical dependence. At the level of these structures there are prostaglandin receptors, EP1, EP2, EP3, FP but also receptors for petidoleukotrienes, cys LT1, cys-LT2. There also is synthesis of prostaglandins and membrane receptors for these [13].

The frequency and intensity of symptoms during the withdrawal syndrome quantify the severity of physical dependence. Chronic exposure to opioids leads to adaptations that either reduce activity of the opioid receptor or produce an upregulation of some neurotransmitters contributing to the symptoms of opioid withdrawal [22]. The neural adaptations include interactions between some neurotransmitters (calcitonin gene-related peptide (CGRP), P substance, endocannabinoid pathways) and among these, signaling messengers derived from the metabolism of arachidonic acid play a leading role, both for cycloxygenase products [7] and for lipoxygenase (LOX)[5].

In both the induction and expression of the opioid tolerant-dependent state several studies have shown an important role for activity of l-glutamate, expressed via spinal N-methyl-d-aspartate (NMDA) receptors. Blockade of NMDA receptor inhibits the expression of naloxone-precipitated morphine withdrawal. In the spinal cord, activation of NMDA receptors induces the synthesis and release of prostaglandins from astrocytes and neurons. Prostaglandins generated from these sources act on primary afferent terminals, modulate the release of excitatory neuromediators [21] and under the influence of chronic morphine, may have significance in the opioid-induced dependence.

The activity of spinal prostaglandins might also be involved in opioid physical dependence. Dunbar et al. have demonstrated that acute intrathecal ibuprofen attenuates the hyperalgesia associated with morphine withdrawal. A number of withdrawal signs, encompassing the sensory, autonomic, and motor components of the opioid withdrawal syndrome, can be partially suppressed by intrathecal administration of COX-2 selective inhibitors (e.g. nimesulide)[22]. A high level of prostaglandin activity may lead to neuropeptide release from spinal sensory neurons during morphine withdrawal. An adaptive increase in the expression or activity of this enzyme in response to chronic opioid treatment may contribute to the genesis of tolerance and dependence [24]. There is evidence involving activity of arachidonate metabolites generated from the LOX [5] in the induction and expression of

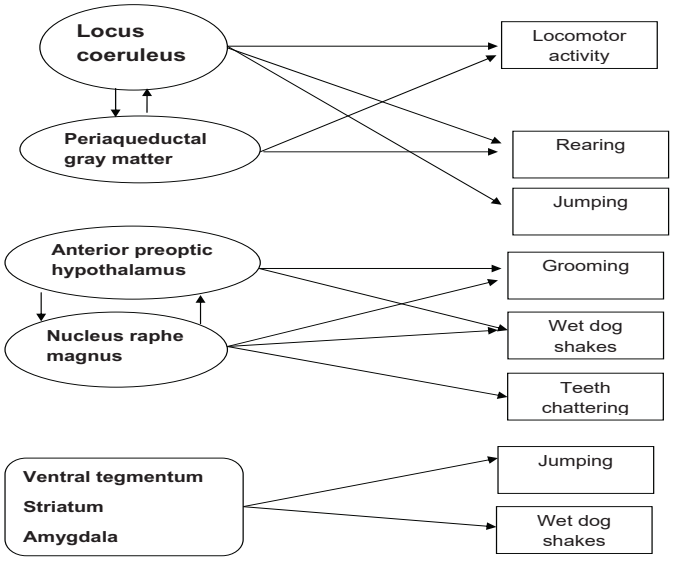

Figure 1 Areas involved in different symptoms of morphine withdrawal (after Maldonado et al[12])

opioid tolerance and physical dependence.

Biochemical and behavioral findings provided the proof of 5- and 12-LOX-derived metabolites' involvement in opioid tolerance and physical dependence. The presence of 12-LOX mRNA has been identified in the spinal cord and opioid treatment significantly induces the expression and activity of this enzyme [18]. Thus, chronic morphine exposure likely increases activity of the LOX cascade at spinal level and inhibition of this cascade at different loci blocks the genesis of the opioid tolerant-dependent state. The effects of LOX inhibitors on opioid tolerance-dependence related to spinal CGRP expression are strikingly similar to those previously seen following intervention with nonselective and COX-2 selective inhibitors [8,19]. This suggests that metabolites derived from both pathways likely contribute to development of the opioid tolerant-dependent state through a common intracellular signaling pathway that directly or indirectly modulates activity of spinal CGRP. There are data that show involvement of prostaglandins (but also other eicosanoids) in presynaptic release of neuromediators like dopamine and serotonin, considered keystones in the emergence of physical dependence.

Natural prostaglandins have the disadvantage of a very short half time. Nowadays, a number of synthetic analogues of $PGF_{2\alpha}$ and PGE are introduced in therapy (human and veterinary). These are more stable than the natural ones and have a longer half-time.

We tested the influence of optically active cloprostenol (ClPGOA) and $PGF_{2\alpha}$ isopropyl ester (IPEF) on experimental morphine- induced physical dependence in rat. The results showed that ClPGOA and IPEF administrated during morphine-induced physical dependence moderately but significantly statistically influenced some of the symptoms of withdrawal syndrome. Not all symptoms were equally influenced. (Table I). Statistically significant influence was noticed on: grooming, aggressive postures, teeth chattering, compulsive mastication explorations. There was no influence noted on: weight loss, diarrhea, jumping [16]. Our results are in accordance with Nielsen and Sparber,[17] which observed an attenuation of opiate-induced physical dependence after $PGF_{2\alpha}$ intracerebroventricular administration in rat.

Group Sign	**Morphine (M) group**	**M+ ClPG 25**	**p vs. M group**	**M+ IPEF 25**	**p vs. M group**	ClPG **25**	**p vs. M group**
Compulsive mastication	39 ± 2	26.3 ± 1.16	< 0.01	23 ± 3	< 0.05	4.65 ± 0.24	< 0.01
Jumpings	10.4± 0.75	8.4 ± 0.36	< NS	7.9 ± 0.2	< NS	0.34± 0.14	< 0.01
Aggressive positions	12.1± 0.4	8.2 ± 0.15	< 0.05	7.6 ± 0.35	< 0.05	0.87± 0.33	< 0.01
Teeth chattering	9.4± 0.35	7.5±0.25	< 0.05	7.2 ± 0. 6	< 0.05	0.14 ± 0.04	< 0.01
Weight loss	3.2± 0.33	3.21 ± 0.3	NS	3.01 ± 0.8	NS	0.85 ± 0.06	< 0.01
Diarrhea	3.4 ± 0.3	2.9± 0.25	NS	3 ±.0.2	NS	0.26 ± 0.01	< 0.01
Eyelid ptosis	4.2±0.18	3.2± 0.04	< 0.05	2.7± 0.08	< 0.05	0.24 ± 0.5	< 0.01
Grooming	37 ±4.5	17 ±2.5	< 0.01	20± 3.45	< 0.01	0.67 ± 0.13	< 0.01

Table I The influence of optically active cloprostenol (ClPGOA) and $PGF_{2\alpha}$ isopropyl ester (IPEF) on morphine (M) – induced withdrawal syndrome (after Nechifor et al [16]).

Nakagawa et al, 1995 [14] have shown that some prostaglandin analogues acting on EP3 receptors attenuated withdrawal jumping in morphine dependent mice but consider that intracerebroventricular administration of $PGF_{2\alpha}$ had no effect in this situation. We didn't observe an influence on jumpings but we observed a significant change of these 2 analogues of $PGF_{2\alpha}$ tested (ClPGOA and IPEF) on other withdrawal signs in morphine- induced dependence.

Another prostaglandin analogue used in human therapy is misoprostol (Mis) (a synthetic, stable PGE_1 analogue). Our data show that Mis (100 and 50 μg/kg i.p. – higher than therapeutic doses, but lower

than toxic doses) administered during acquisition of morphine-induced physical dependence significantly decreases, in a dose-dependent manner, some of the symptoms from withdrawal syndrome: compulsive mastication, jumpings, aggressive positions and penile erection [e.g jumpings from 8.7±0.45 in morphine (M) group to 1.3±0.066 in M +Mis 100 ($p<0.01$); 4±0.2 in M+Mis 50 ($p<0.01$) and 7.6±0.4 in M+Mis 5 (NS)].

PGE_2 has a modulating role upon dopaminergic neurotransmission in some CNS areas involved in the reward system, by stimulation of EP_2 and EP_3 receptors[6]. EP_3 receptors are also present in (especially presynaptic) neuronal membrane, and are involved in catecholamine release in the brain. Their stimulation decreases presynaptic release of neurotransmitters [10].Stimulation of EP_3 receptors decreases dopamine release not only in the CNS but also in other body regions [15]. There also is a postsynaptic action of PGEs in many brain areas.

Our data have shown a significant decrease of some symptoms from withdrawal syndrome by Mis (agonist on EP_2/EP_3). We consider that this action is due to decrease in dopaminergic mediation in the reward system (the main system involved in morphine-induced physical dependence). Our data are in agreement with Nakagawa et al (2000) which have shown that EP_3 stimulation with EP_3 agonist MB28767 leads to decreasing withdrawal syndrome in rats with morphine–induced dependence[13].

The influence of leukotrienes in morphine-induced physical dependence is less clear. Data of Rehni et al, 2008 [20] have shown that montelukast (a leukotriene LT1 receptor antagonist) has an influence in morphine-induced physical dependence in mice. Using a different experimental setup in rats, our preliminary data partly confirm an influence of montelukast (MLK) in morphine(M)-induced physical dependence, only a few symptoms being influenced (e.g. locomotor activity 1.14 ± 0.2 in M+MLK group vs 1.57 ±0.1 in M group, $p< 0.05$)

The development of tolerance and physical dependence following chronic exposure to opioids involves multiple mechanisms that lead to an adaptive increase in spinal pronociceptive neuropeptides. Activity of transmitters such as CGRP, substance P and their receptors, expressed via signaling messengers (prostaglandins, LOX products and endocannabinoids) contributes to the loss of opioid analgesic potency and expression of the opioid withdrawal syndrome. The nature of the interaction between these different factors, and the cellular-molecular mechanisms through which their activity leads to the induction of the opioid tolerance remain unclear and request further investigation. However, pharmacological interventions that block activity or inhibit production of these factors may have the potential of maintaining the analgesic actions of opioids and preventing the development of physical dependence associated with chronic opioid treatment.

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