

## Research Article

# Peripheral Antinociception Induced by Aripiprazole Is Mediated by the Opioid System

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**Background.** Aripiprazole is an antipsychotic drug used to treat schizophrenia and related disorders. Our previous study showed that this compound also induces antinociceptive effects. The present study aimed to assess the participation of the opioid system in this effect. **Methods.** Male Swiss mice were submitted to paw pressure test and hyperalgesia was induced by intraplantar injection of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>, 2 µg). Aripiprazole was injected 10 min before the measurement. Naloxone, clocinnamox, naltrindole, nor-binaltorphimine, and bestatin were given 30 min before aripiprazole. Nociceptive thresholds were measured in the 3rd hour after PGE<sub>2</sub> injection. **Results.** Aripiprazole (100 µg/paw) injected locally into the right hind paw induced an antinociceptive effect that was blocked by naloxone (50 µg/paw), a nonselective opioid receptor antagonist. The role of µ-, δ-, and κ-opioid receptors was investigated using the selective antagonists, clocinnamox (40 µg/paw), naltrindole (15, 30, and 60 µg/paw), and nor-binaltorphimine (200 µg/paw), respectively. The data indicated that only the δ-opioid receptor antagonist inhibited the peripheral antinociception induced by aripiprazole. Bestatin (400 µg), an aminopeptidase-N inhibitor, significantly enhanced low-dose (25 µg/paw) aripiprazole-induced peripheral antinociception. **Conclusion.** The results suggest the participation of the opioid system via δ-opioid receptor in the peripheral antinociceptive effect induced by aripiprazole.

## 1. Introduction

Aripiprazole is an antipsychotic drug with a complex pharmacology. Its main mechanism of action consists in the partial agonism at dopamine D<sub>2</sub> receptor [1]. In investigating the behavioral pharmacology of aripiprazole in experimental animals, we showed that this compound also decreases the PGE<sub>2</sub>-induced hyperalgesia in a dose-dependent manner in the mechanical paw withdrawal test [1]. In addition, systemic administration of this compound reduced the licking time in the second phase of the formalin test and in the latency time of tail flick test [2].

Recently, it was demonstrated that neurons expressing the dopaminergic receptors were immunopositive for the endogenous opioid met-enkephalin [3]. Met-enkephalin is produced after the cleavage of precursor peptide proenkephalin (PENK) protein. Other endogenous opioids include endorphin, formed after the cleavage of the precursor proopiome-lanocortin (POMC) protein, and dynorphins derived from

cleavage of prodynorphin. Endogenous opioids may bind preferentially to one of the three opioid receptors. Enkephalin has higher affinity for µ opioid receptors, whereas endorphin binds to µ and δ-opioid receptors and prodynorphin exhibits higher affinity for opioid κ-receptors [4]. Opioid receptors are metabotropic receptor coupled to Gi protein. Once activated by agonists, such as morphine or endogenous opioid peptides, they lead to the inhibition of adenylate cyclase and reduction of cAMP synthesis [5]. They also impair extracellular calcium influx and inhibit cell depolarization [6–8].

The opioid system is widely distributed in the peripheral and central nervous system (CNS) [9]. They have been implicated in peripheral antinociception induced by nonopioidergic compounds, including nonsteroidal anti-inflammatory drugs [10] and α<sub>2</sub>-adrenergic agonists [11–13]. Therefore, the peripheral study could be a tool to minimize side effects and to facilitate drugs administration. Thus, considering this

context, the aim of the present study was to test the hypothesis that opioid receptors mediated the antinociceptive effect of aripiprazole.

## 2. Materials and Methods

**2.1. Animals.** The present study was approved by the Committee for Ethics in Animal Experimentation (CEUA) under the protocol number 109/2011. Every effort was made to minimize any suffering of animals. All experiments were performed on 30–35 g (aged: 3 months) male Swiss mice and were kept in a cage of size 30 × 35 cm, with 10 animals in each cage in a room maintained at 25 ± 1°C with a 12 h light/dark cycle (6:00 a.m.–6:00 p.m.). Each animal was used only once. Food and water were available ad libitum.

**2.2. Drugs.** Aripiprazole was injected subcutaneously into the plantar surface of the right hind paw (25 and 100 µg). The substance was provided by Bristol-Myers Squibb (Syracuse, NY, USA) and Otsuka Pharmaceuticals (Naruto, Tokushima, Japan). It was dissolved in physiological saline containing 5% tween 80; prostaglandin E<sub>2</sub> (PGE<sub>2</sub>; hyperalgesic agent; Sigma®) was diluted in ethanol [14]. All other drugs used were diluted in saline, naloxone (nonselective antagonist at opioid receptors, Sigma), bestatin (an aminopeptidase-N inhibitor, Tocris®), clocinnamox (selective µ-opioid receptor antagonist, Sigma), nor-Binaltorphimine dihydrochloride (nor-BNI, selective κ-opioid receptor antagonist, Sigma), and naltrindole (selective δ-opioid receptor antagonist, Tocris).

**2.3. Measurement of Hyperalgesia.** Hyperalgesia was induced by subcutaneous injection of PGE<sub>2</sub> (2 µg) into the plantar surface of the right hind paw and it was measured according to the paw pressure test described by Randall and Selitto [14] and modified by Kawabata and coworkers [15]. An analgesiometer (Ugo-Basile, Italy) with a cone-shaped paw-presser with a rounded tip was used to apply a linearly increasing force to the right hind paw of the mice. The weight in grams required to elicit a nociceptive response, the paw withdrawal threshold, was determined as the nociceptive threshold. A cutoff value of 160 g was used to reduce the possibility of damaging the paw. The nociceptive threshold was measured in the right paw and determined by the average of three consecutive trials recorded before (zero time) and 3 hours after PGE<sub>2</sub> injection (peak of action). The results were calculated by the difference between these two averages (Δ of nociceptive threshold) and expressed in grams. To reduce stress, the mice were habituated to the apparatus 1 day prior to the experiments.

**2.4. Experimental Protocols.** In all experiments the baseline threshold of each animal was first determined before the injection of any substance. PGE<sub>2</sub> (2 µg) was given and the nociceptive responses were measured after 180 minutes. To evaluate the antinociceptive peripheral effects of aripiprazole, this compound (25 or 100 µg) was given into the right paw 170 min after PGE<sub>2</sub> injection. To test if its effects would be inhibited by naloxone (50 µg), naltrindole (15, 30, and 60 µg),

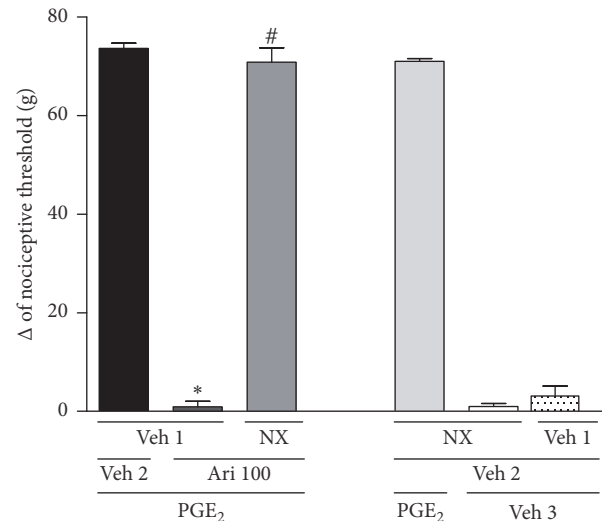


FIGURE 1: Naloxone antagonizes aripiprazole-induced antinociceptive effect against the hyperalgesic effect induced by PGE<sub>2</sub> (PGE<sub>2</sub>, 2 µg). Naloxone (NX; 50 µg/paw) and aripiprazole (Ari; 100 µg/paw) were given 140 min and 170 min after PGE<sub>2</sub>. The data are presented as mean and SEM (\**p* < 0.05 compared with the PGE<sub>2</sub> + Veh 1 + Veh 2; #*p* < 0.05 compared with the PGE<sub>2</sub> + Veh 1 + aripiprazole 100 µg group; ANOVA followed by the Bonferroni test; *n* = 4 per group).

clocinnamox (40 µg), or nor-BNI (200 µg), these antagonists were given 140 min and aripiprazole (100 µg) 170 min after PGE<sub>2</sub> injection. To investigate the effects of an aminopeptidase-N inhibitor, bestatin (400 µg) was administered 140 min prior to PGE<sub>2</sub>. The protocols concerning dose and time of administration of each drug used in this study were obtained through pilot experiments and literature data [16, 17].

**2.5. Statistical Analysis.** The data were analysed with the GraphPad Prism 5 Software®. Drug treatments were compared by one-way analysis of variance (ANOVA). Post hoc analyses were performed with the Bonferroni test. All data are expressed as the mean and SEM statistical difference was set as *p* < 0.05.

## 3. Results

The injection of aripiprazole (100 µg/paw) into the right hind paw produced an antinociceptive response against PGE<sub>2</sub>-induced hyperalgesia (2 µg/paw, Figure 1). To verify the involvement of opioid system in this effect, the mice were pretreated with nonselective opioid receptor antagonist naloxone. Naloxone (50 µg/paw) antagonized peripheral antinociceptive response of aripiprazole (100 µg/paw) [*F*<sub>(4,15)</sub> = 553.8; *p* < 0.0001]. When injected alone, naloxone did not induce antinociception or inhibit PGE<sub>2</sub>-induced hyperalgesia.

Once the involvement of opioid receptors in the mechanism of aripiprazole-induced antinociception was confirmed, the next step was to assess specifically which opioid receptor was involved in this process. The µ-, κ-, and δ-opioid

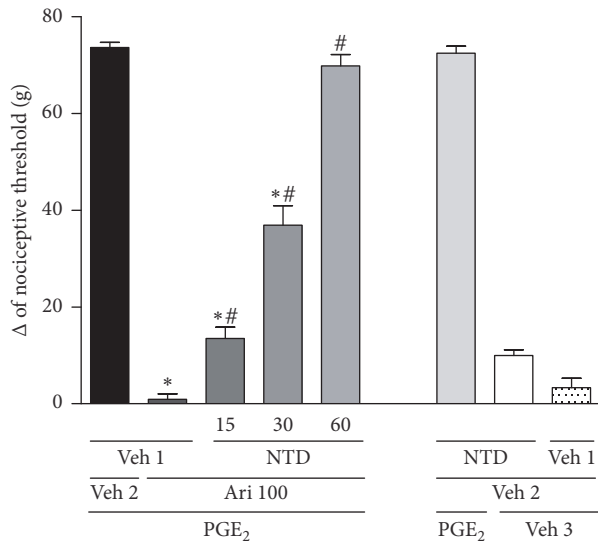


FIGURE 2: Naltrindole antagonizes the aripiprazole-induced antinociceptive effect against the hyperalgesic effect induced by PGE<sub>2</sub> (PGE<sub>2</sub>, 2 μg). Naltrindole (NTD; 15, 30, and 60 μg/paw) and aripiprazole (Ari; 100 μg/paw) were given 140 min and 170 min after PGE<sub>2</sub>. The data are presented as mean and SEM (\**p* < 0.05 compared with the PGE<sub>2</sub> + Veh 1 + Veh 2; #*p* < 0.05 compared with the PGE<sub>2</sub> + Veh 1 + aripiprazole 100 μg group; ANOVA followed by the Bonferroni test; *n* = 4 per group).

receptor antagonists, clocinnamox (40 μg/paw), nor-BNI (200 μg/paw), or naltrindole (15, 30, and 60 μg/paw), were injected prior to aripiprazole (100 μg/paw, the dose required to reverse almost 100% of nociception). Naltrindole was able to inhibit the antinociceptive effect induced by aripiprazole in a dose-dependent manner [ $F_{(6,21)} = 247.4$ ; *p* < 0.0001], Figure 2. However, neither clocinnamox [ $F_{(4,15)} = 397.1$ ; *p* < 0.0001] nor nor-BNI [ $F_{(4,15)} = 380.9$ ; *p* < 0.0001] blocked the antinociceptive response of aripiprazole, Figures 3 and 4. None of the compounds affected the nociceptive effect of PGE<sub>2</sub> by themselves.

To evaluate the involvement of endogenous opioid peptides in the antinociceptive effect mediated by aripiprazole, the animals were treated with intraplantar injection of bestatin (400 μg/paw). This administration increased the peripheral antinociceptive effect of aripiprazole (25 μg/paw) [ $F_{(4,15)} = 331.5$ ; *p* < 0.0001], the dose required to induce about 50% of antinociception, Figure 5. Bestatin alone did not affect the nociceptive effect of PGE<sub>2</sub>.

#### 4. Discussion

This study evaluated the mechanisms of peripheral antinociception induced by aripiprazole, an antipsychotic drug that acts as a partial agonist at dopamine D<sub>2</sub> receptor. The increased nociceptive response was induced by PGE<sub>2</sub>, which sensitizes primary afferent neurons and provokes hyperalgesia to a mechanical stimulus [18]. Previous work showed that aripiprazole prevented PGE<sub>2</sub> effects in this model through activation of dopamine D<sub>2</sub> and serotonin 5-HT<sub>1A</sub>

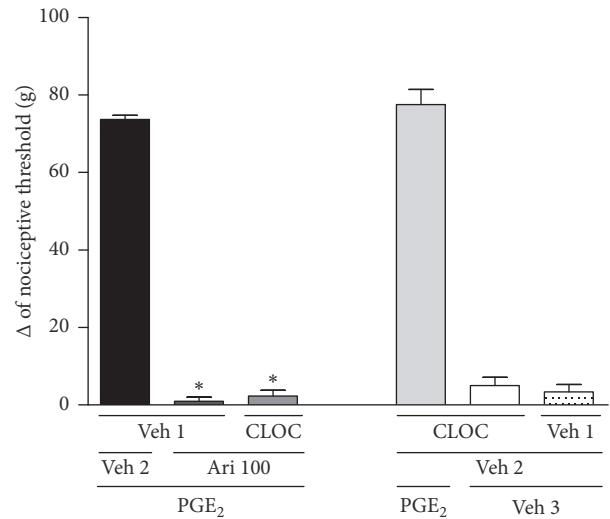


FIGURE 3: Clocinnamox did not antagonize the aripiprazole-induced antinociceptive effect against the hyperalgesic effect induced by PGE<sub>2</sub> (PGE<sub>2</sub>, 2 μg). Clocinnamox (CLOC; 40 μg/paw) and aripiprazole (Ari; 100 μg/paw) were given 140 min and 170 min after PGE<sub>2</sub>. The data are presented as mean and SEM (\**p* < 0.05 compared with the PGE<sub>2</sub> + Veh 1 + Veh 2; ANOVA followed by the Bonferroni test; *n* = 4 per group).

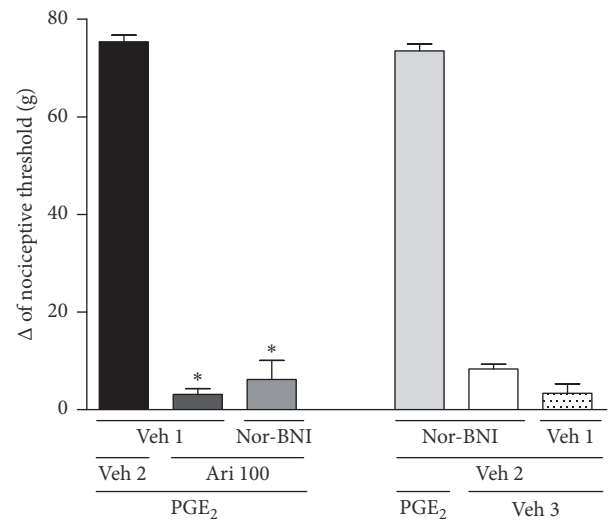


FIGURE 4: Nor-binaltorphimine did not antagonize the aripiprazole-induced antinociceptive effect against the hyperalgesic effect induced by PGE<sub>2</sub> (PGE<sub>2</sub>, 2 μg). Nor-binaltorphimine (nor-BNI; 200 μg/paw) and aripiprazole (Ari; 100 μg/paw) were given 140 min and 170 min after PGE<sub>2</sub>. The data are presented as mean and SEM (\**p* < 0.05 compared with the PGE<sub>2</sub> + Veh 1 + Veh 2; ANOVA followed by the Bonferroni test; *n* = 4 per group).

receptors [1]. However, considering the complex mechanisms modulating nociceptive processing, we do not rule out the possibility that additional mechanisms might contribute to the antinociceptive effect of aripiprazole, for example, the opioid system.

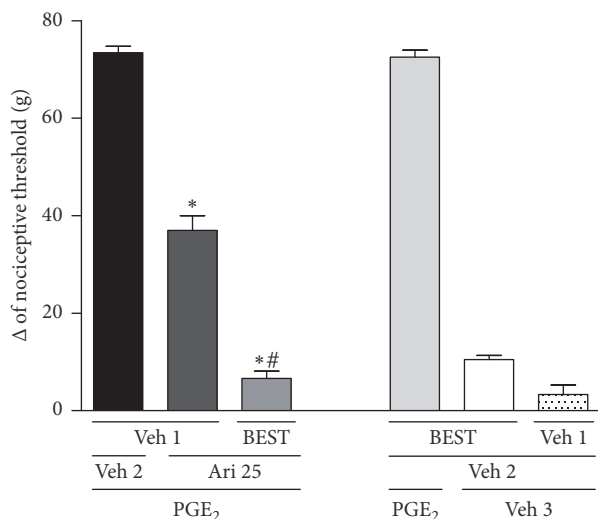


FIGURE 5: Bestatin potentiates the aripiprazole-induced antinociceptive effect against the hyperalgesic effect induced by PGE<sub>2</sub> (PGE<sub>2</sub>, 2 μg). Bestatin (BEST; 400 μg/paw) and aripiprazole (Ari; 25 μg/paw) were given 140 min and 170 min after PGE<sub>2</sub>. The data are presented as mean and SEM (\**p* < 0.05 compared with the PGE<sub>2</sub> + Veh 1 + Veh 2 group; #*p* < 0.05 compared with the PGE<sub>2</sub> + Veh 1 + aripiprazole 25 μg group; ANOVA followed by Bonferroni test; *n* = 4 per group).

Opioids exert their effects through the Gi protein-coupled receptors  $\mu$ ,  $\delta$ , and  $\kappa$  [19]. Their antinociceptive effects are well-established in different animal models, such as formalin [20–22] and tail flick [2, 23] tests.

In this work, naloxone, a nonselective opioid receptor antagonist, inhibited the peripheral antinociception induced by aripiprazole. The role of the  $\mu$ -,  $\delta$ -, and  $\kappa$ -opioid receptors was investigated using their selective antagonists clocinnamox, naltrindole, and nor-binaltorphimine, respectively. Our data indicated that only  $\delta$ -opioid antagonist was able to reverse the peripheral antinociception induced by aripiprazole. This result is in agreement with several studies suggesting a role of  $\delta$ -opioid receptor in peripheral antinociceptive effects [24–26]. Izquierdo and coworkers demonstrated that the peripheral administration of mangiferin produced a reduction of nociception in response to the formalin test, mediated by  $\delta$ -receptors peripherally [26]. In addition,  $\delta$ -receptors also mediated peripheral antinociception of the potent analgesic peptide, crotalphine, in a model of cancer pain induced by intraplantar injection of Walker 256 carcinoma cells [27]. In line with these data, the  $\delta$ -opioid receptor agonist, SNC80, induced peripheral antinociceptive effect [28, 29]. Finally, PnPP-19, a spider toxin peptide, induces peripheral antinociception through  $\delta$ -opioid receptor in rats [30]. Altogether, these results support our findings that aripiprazole induces peripheral antinociceptive effects through facilitation of the opioid system, particularly the  $\delta$ -opioid receptor.

In the CNS, opioid receptors are expressed in subcortical regions of the brain (thalamus, cerebral cortex, periaqueductal grey, rostral ventromedial medulla, and amygdala, among others), from which descending pain-modulating pathways

originate, and also in the dorsal horn of the spinal cord, an important area that sends nociceptive inputs to the brain and also a primary action site for opioids analgesic effects [9, 31–34]. In addition to this, at the peripheral level, the opioid receptors are expressed not only in neuronal cells [35, 36], but also in immune cells (macrophages and neutrophils) as well as keratinocytes [37]. Similarly, D<sub>2</sub> receptors are also expressed at significant levels in the nucleus accumbens, ventral tegmental area, hypothalamus, cortical areas, septum, amygdala, and hippocampus [38–41]. Moreover, D<sub>2</sub> and 5-TH<sub>1A</sub> receptors are also found in dorsal root ganglia (DRG) and keratinocytes, showing the peripheral presence of these receptors [42–45].

It remains unclear, however, how aripiprazole facilitates the endogenous opioid system. D<sub>2</sub> receptor might interact with the opioid system at a downstream level, such as by facilitating  $\delta$ -opioid and D<sub>2</sub> receptor heterodimerization or by interfering with signal transduction processes. Neurochemical works have shown that dopamine and opioid systems are one of the major endogenous systems involved in several behaviors, such as pain perception and its modulatory mechanisms, the reward system, dependence, and fear control [1, 2, 46–48]. Furthermore, previous studies showed that the systemic administration of  $\delta$ -opioid receptor agonists facilitates dopaminergic activity in the striatum and forebrain regions, as determined by high-affinity dopamine uptake and turnover rates [49, 50]. Le Moine and coworkers demonstrated that the major striatopallidal neurons express D<sub>2</sub> receptors and enkephalin [51], suggesting an interaction between the dopamine and opioid systems. It remains to be investigated if these mechanisms also operate to modulate pain responses in the periphery.

Another possibility is that D<sub>2</sub> receptor partial agonist facilitates the release of endogenous opioids which, in turn, activate the  $\delta$ -opioid receptor. The result showing that bestatin, an aminopeptidase-N inhibitor, potentiated the peripheral antinociceptive effect induced by a low dose of aripiprazole supports this possibility. D<sub>2</sub> receptor could interact with the beta-gamma complex ( $G\beta\gamma$ ) signaling and activate phospholipase C (PLC). This would lead to IP<sub>3</sub> receptor activation, resulting in increase in intracellular calcium [52, 53]. Calcium increase would stimulate the synthesis of proenkephalin (PENK) which, in turn, activates the  $\delta$ -opioid receptor to reduce nociceptive response.

In conclusion, our data suggest that the peripheral antinociceptive effect of aripiprazole is associated with facilitation of endogenous opioid activity through the  $\delta$ -opioid receptors. The therapeutic potential of aripiprazole for the treatment of certain types of pain warrants further investigation.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

## Authors' Contributions

Renata Cristina Mendes Ferreira and Ana Flávia Almeida-Santos contributed equally to this work.

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