# THE CENTRAL ANTINOCICEPTIVE EFFECT OF N-ARACHIDONOYL-PHENOLAMINE (AM404): IMPLICATION OF OPIOIDERGIC AND SEROTONERGIC SYSTEMS.

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Recently it has been demonstrated that paracetamol (PARA) undergoes a metabolic transformation to form N-arachidonoyl-phenolamine (AM404) that is a potent agonist of $TRPV_1$, a ligand at $CB_1$ receptors, and an inhibitor of cellular anandamide uptake (1). Cannabinoids produce antinociceptive effects in several animal models that are mediated chiefly by $CB_1$ receptors. The cannabinoid-induced antinociception seems to depend, to some extent, also on the release of opioid peptides into the brain and on the activation of μ receptors. Moreover, the antinociceptive activity of AM404 is antagonized by $CB_1$ receptor antagonists. We previously demonstrated that the antinociceptive effect of PARA matches an increase in serotonin levels and a decrease in the number of 5-$HT_2$ receptors in the cerebral cortex and in the pons of the rat. Moreover, the PARA-induced antinociception may be partially mediated by the opioidergic system in the brain. The present work was aimed at detecting whether AM404 acts through the same mechanisms by which PARA exerts its analgesic effect, mainly serotonergic and opioidergic. We firstly investigated the possible role of the opioidergic system in the antinociceptive effect of AM404 (10 mg/kg i.p.) using naloxonazine (a selective $\mu_1$ receptor antagonist, 10 mg/kg i.p., 24 h before AM404). A mutual correlation between the serotonergic and opioidergic neurotransmitter systems has been detected in the pain control pathways. Secondly we assessed the implication of serotonergic system in the AM404-induced antinociceptive effect using: a) 5-$HT_{1A}$ (NAN-190 5 mg/kg i.p.), 5-$HT_2$ (ketanserin 5 mg/kg s.c.) and 5-$HT_3$ (ondansetron 2 mg/kg s.c.) receptor antagonists, injected 15 min before AM404; b) evaluating possible changes in 5-HT and 5-HIAA levels in the frontal cortex and in the pons of the rat. The hot-plate test (1 h after the last treatment) was used to evaluate pain threshold and HPLC for biochemical determinations. The antinociceptive effect of AM404 was about 40% lesser than that of PARA (%MPE values: CTRL=0.45±1.1; AM404=14.14±3.2; PARA=29.29±4.3, means±E.S.M.; ANOVA followed by Bonferroni test) and was completely prevented by both naloxonazine and ondansetron while NAN-190 and ketanserin were ineffective. 5-HT levels were slightly increased, while 5-HIAA ones remained unchanged after AM404 treatment. We conclude that the mechanisms of the antinociceptive effect of AM404 and PARA differs in some way, suggesting that this biotransformation did not completely explain the mechanism by which PARA works.

(1) Flegley D. et al. (2004) Proc. Natl. Acad. Sci. U.S.A. 101: 8756-61.