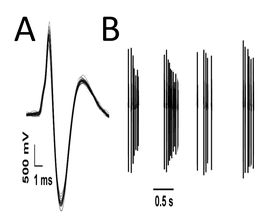
INHIBITION OF ANANDAMIDE HYDROLYSIS DIFFERENTIALLY MODULATES COCAINE-INDUCED EFFECTS ON RAT MESOLIMBIC SYSTEM

Antonio Luchicchi, Salvatore Lecca, Stefano Carta, Giuliano Pillolla and Marco Pistis

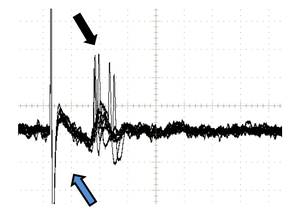
"B.B. Brodie" Department of Neuroscience, University of Cagliari, Italy

RATIONALE - The fatty acid amide hydrolase (FAAH) is the main enzyme which inactivates the endocannabinoid anandamide (AEA) and other n-acylethanolmines (NAEs). Recently, it was demonstrated that inhibition of FAAH suppresses nicotine-induced effects within the brain reward circuitry (7; 8; 9). For this reason, we asked whether FAAH inhibition by URB597 could also modulate the effect of other drugs of abuse such as cocaine within the mesoaccumbens pathway. To this aim we carried out single unit electrophysiological recordings in anaesthetized rats in ventral tegmental area dopamine (VTA DA) neurons and GABAergic medium spiny neurons (MSNs) of the shell of the nucleus accumbens (ShNAc). .

MATERIALS AND METHODS - Male Sprague Dawley rats (Harlan, Italy) were used in all experiments. Animals were anaesthetized with urethane (1.3 g/kg, i.p.) and their femoral vein was cannulated for intravenous (i.v.) administration of pharmacological agents. The scalp was retracted and a burr hole was drilled for the placement of a recording electrode above the VTA (AP +2.0 mm from Lambda; L 0.3-0.5 from the midline). Two burr holes were drilled for carrying on experiments on the ShNAc : one above the ShNAc (AP +1.5 mm from bregma, L 0.8-1.2 mm from midline) for the placement of the recording electrode, and the other above the basolateral amigdala (BLA) (AP -5.8 mm from bregma, L 5 mm from midline) for the placement of the stimulant electrode (15° of inclination in the sagittal axis). A 1 Hz stimulation driven by BLA was applied to evoke spike firing of MSNs. The protocol was already published for VTA experiments (3; 4; 5; 10) as well as for the NAc experiments (1). Only one cell was recorded per rat

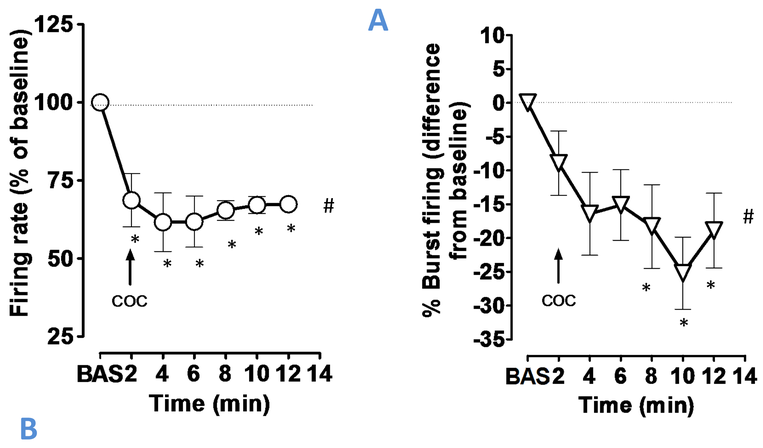


A) Superimposed traces, acquired from a digital storage oscilloscope, showing the spontaneous firing of a VTA DA neuron recorded from a anaesthetized rat.
(B) Representative traces displaying a typical recording from a VTA DA neuron.



Single pulse stimulation (1 Hz) of the BLA evoked firing activity of neurons in the ShNAc. Digital storage oscilloscope traces displaying superimposed traces demonstrating a relatively constant latency of the orthodromic responses (black arrow). The blue arrow indicates the stimulus artifact.

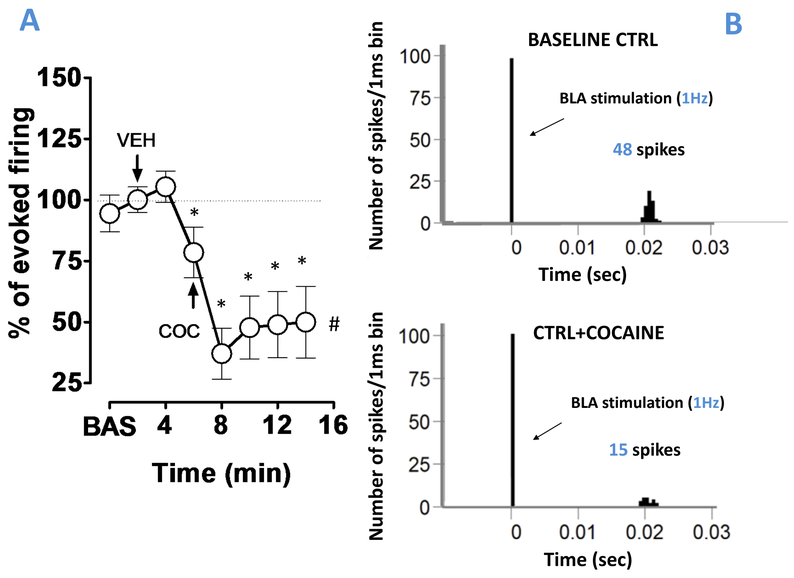
COCAINE INHIBITS FIRING RATE AND BURST FIRING OF VTA DA NEURONS



A. Cocaine (1 mg/kg, i.v.) inhibits firing rate (n=6, 67.1±10% of baseline firing rate, P<0.01, one-way ANOVA # and Dunnet's test) and burst firing (n=6, -10±6% of baseline burst firing, P<0.01, one-way ANOVA# and Dunnet's test*) of VTA DA neurons. Cocaine injection (arrow) is preceded by the administration of vehicle (1 ml/kg, i.v.) which does not affect firing rate or burst firing.*

B. Representative firing rate histograms of individual VTA DA neurons recorded from a naive animal, pre and post cocaine administration

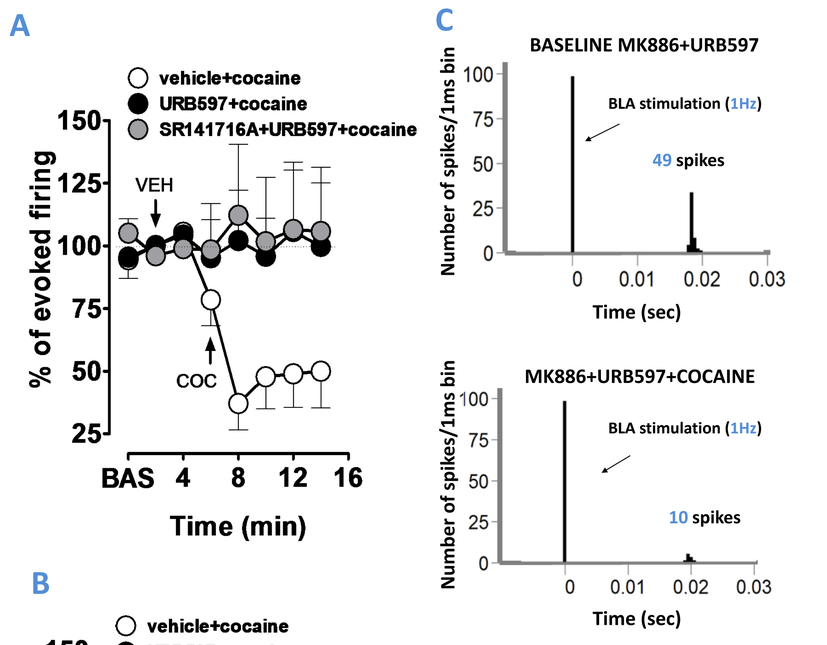
COCAINE INHIBITS THE EXCITABILITY OF MSNs OF THE ShNAc



A. Cocaine (1 mg/kg i.v.) persistently inhibits the excitability of shNAc MSNs (n=6, 37.2±10.6% of baseline level, P < 0.01, one-way ANOVA# and Dunnet's test), as measured by their response to threshold BLA stimulation.*

B. Typical peristimulus time-histograms which show the probability to evoke a MSNs action potential in response to BLA stimulation before and after cocaine administration.

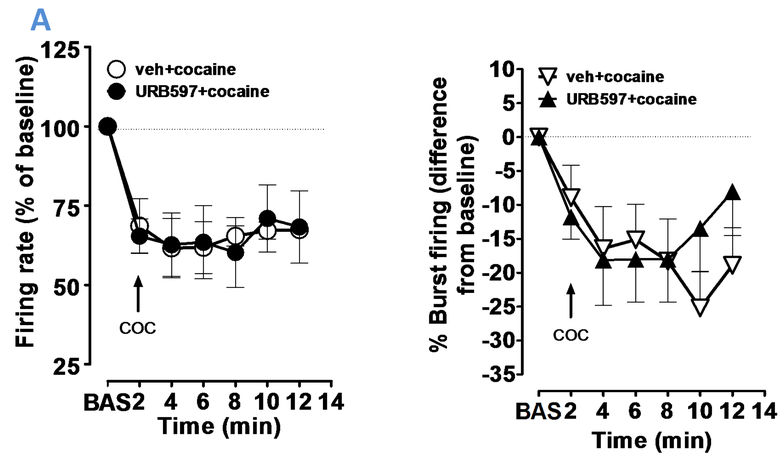
THE EFFECTS OF URB597 ON COCAINE-INDUCED INHIBITION OF MSNs ARE MEDIATED BY PEROXISOME-PROLIFERATOR ACTIVATED RECEPTOR TYPE α (PPAR-α)



A. The effect of URB597 is not abolished by SR (0.5 mg/kg i.v.) pretreatment (n=6; 98.34±18.45% of baseline P>0.05, vs. URB597, two-way ANOVA and Dunnet's test), suggesting a mechanism of action which does not involve the activation of cannabinoid receptors type 1 (CB-1 Rs) by increased levels of AEA.
Since URB597 not only elevates the level of AEA but also the level of other NAEs, like oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) (2) natural ligands of PPAR-α we pretreated animals with the PPAR-α antagonist MK-886 (3 mg/Kg i.p., 15 minutes before URB597).
B. Following MK-886 pretreatment, URB597 effect is completely prevented and cocaine exerts an inhibitory effect similar to control condition (n=6, 58.02±15.59% of baseline, P<0.05, vs. URB597, two-way ANOVA # and Bonferroni's test)

B. Illustrative peristimulus time-histograms which show the probability to evoke a MSNs action potential in response to BLA stimulation before and after cocaine administration in MK886+URB597 pretreated animals

URB597 DOES NOT REVERT COCAINE EFFECT ON VTA DA

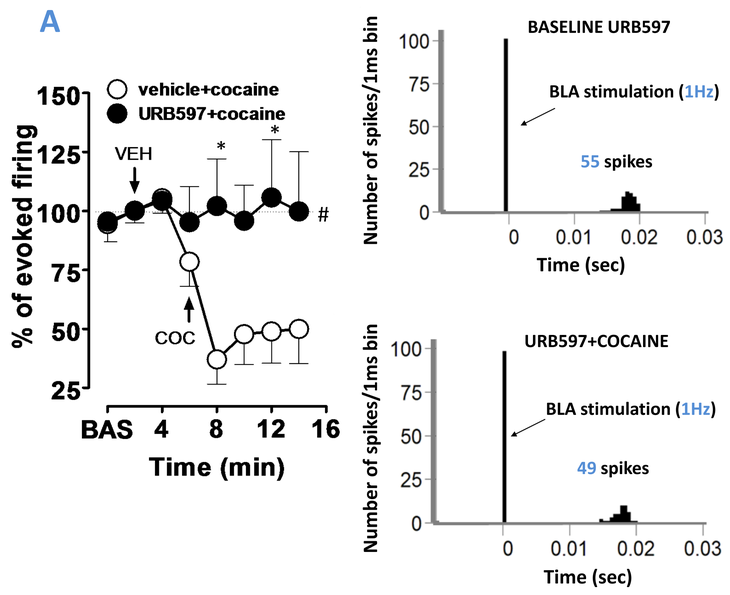


A. Pretreatment with the irreversible FAAH inhibitor URB597 (0.1 mg/kg, i.v. 1-2 h before recordings, n=6) did not prevent the inhibition of DA neurons evoked by intravenous cocaine administration (1 mg/kg). Following URB597 administration, cocaine inhibited firing rate (n=6, 58.9±2.9% of baseline firing rate, P>0.05 vs. control, two-way ANOVA and Bonferroni's test) and burst firing (n=6, -12.9±5.8% of baseline burst firing, P>0.05 vs. control, two-way ANOVA and Bonferroni's test) of DA neurons.

URB597 per se did not induce significant changes in spontaneous firing rate or pattern of DA neurons. Mean firing rates were 3.4 ±0.8 Hz (n=6) and 4.2±0.67Hz (n=6) and percent of spikes in bursts were 20.6±17.50% (n=6) and 27.6 ±11.04% (n=6) for controls and URB597-treated animals , respectively. (P>0.05) (data not shown).

B. Representative firing rate histogram of individual VTA DA neurons recorded from a URB597 pretreated animal

URB597 FULLY PREVENTS COCAINE EFFECTS ON MSNs OF THE ShNAc



A. Following inhibition of the major hydrolyzing enzyme for eCbs (FAAH) by URB597 pretreatment (1-2 hours before recording), cocaine does not exert its inhibitory action on MSNs (n=6, 94.5±18.5%, P<0.05 vs. control, two-way ANOVA# and Bonferroni's test) . URB597 per se does not affect the basal BLA-evoked spike firing of MSNs. The mean current to evoke MSN spiking was 1.59±0.15 vs 1.90±0.42 mA for pretreated-and control rats, respectively (P>0.05, Student's T test)*

B. Peristimulus time-histogram showing the probability to evoke a MSNs action potential in response to BLA stimulation before and after cocaine administration in URB597 pretreated animals

CONCLUSIONS - These findings show that:

- Cocaine inhibits firing rate and burst firing of VTA DA neurons and depresses the excitability of MSNs of the ShNAc
- URB597 fully reverts cocaine effects in the NAc but not in the VTA
- URB597 effects on cocaine-induced inhibition is mediated by the activation of PPAR-α

These data support the hypothesis of a modulatory effect of the elevation of the eCb tone within different areas of the brain reward circuit. Interestingly, URB597 effects is not dependent on CB-1 Rs activation by elevated levels of AEA but it occurs through the activation of PPAR-α. A parsimonious explanation of these results might involve a direct action of NAEs, whose levels are elevated by URB597 (2), on cholinergic (Ach) transmission within the NAc. This hypothesis is supported by other studies which demonstrated an increase in Ach release after psychostimulants administration in the ventral striatum (6). Furthermore, it has been shown that OEA and PEA may be cholinergic modulator themselves, given that their biosynthesis is increased after stimulation of muscarinic receptors (mAChRs) (10) . This active role of PPARs might contribute to modulate cocaine-induced primary reinforcing properties and reward seeking behavior.

REFERENCES

1 Floresco SB. et al.(2001) *J. Neurosci* 21:2851–2860
2 Fegley D. (2005).*J.Pharmacol.Exper.Ther.* 313(1): 352-8
3 Grace AA. and Bunney BS. (1983) *Neuroscience*. 10: 301-315
4 Grace AA. and Bunney BS. (1984) *J neurosci*. 4: 2877-2890
5 Guyenert PG. and Aghajnian GK. (1978) *Brain Res.* 150: 69-84
6 Imperato A. et al. (1992) *Eur. J. Pharmacol.* 229:265-7
7 Luchicchi A. et al. (2009) ECNP workshop suppl. S84
8 Melis M. et al. (2008) *J.Neurosci.* 28: 13985-94
9 Scherma M. et al. (2008) *J.Pharmacol. Exper. Ther.* 327: 482-90
10 Stella N. and Piomelli D.(2001) *Eur. J. Pharmacol.* 425: 189-96
11 Ungless MA. et al. (2004). *Science*. 303: 2040-2042