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## FATTY ACID AMIDE HYDROLASE INHIBITOR IMPROVES FUNCTION OF INFLAMED BLADDERS BY SIMULTANEOUS MODULATION OF ANANDAMIDE AND PALMITOYLETHANOLAMIDE LEVELS.

### Hypothesis / aims of study

The inhibition of fatty acid amide hydrolase (FAAH), the key enzyme of fatty acid amide degradation, is a promising tool for the treatment of bladder hyperactivity and pain related with cystitis. Inhibition of FAAH was hypothesised to increase anandamide (AEA) levels in the bladder wall, thus increasing the activity of cannabinoid receptors which is expected to counteract TRPV1 activity [1]. However, measurements of fatty acid amides after FAAH inhibitors administration to normal and inflamed bladders were never carried out to fully validate this hypothesis.

In the present work we aimed to study the effect of a FAAH antagonist in the inflammation-induced bladder hyperactivity and on the levels of fatty acid amides. AEA acts on CB1 at low levels and on TRPV1 at high levels while palmitoylethanolamide (PEA) acts both on CB1 and TRPV1).

### Study design, materials and methods

Female Wistar rats were divided in lipopolysaccharide (LPS) inflamed group, and control group. For LPS inflammation, rats were intravesical instilled under anaesthesia with 5 mg/ml LPS, for 1 hour. Experiments were then carried out 24 hours after LPS instillation. Bladder inflammation was confirmed by observation of histological signs of cystitis. Control group were intravesical instilled with saline.

The following drugs were used: URB 937 (FAAH antagonist), MJ15 (CB1 receptor antagonist), SR144528 (CB2 antagonist) and SB366791 (TRPV1 antagonist).

Animals belonging to the LPS and control groups received intravenously (caudal vein) during cystometry:

- URB 937 in doses of 0.007, 0.07, 0.7 and 7 mg/kg (cumulative, with 10 minutes interval);
- At the maximal effective dose of URB937 (0.7 mg/kg, see below) animals received 10 microM MJ15 to block CB1 receptor or 0.3 mg/kg SR144528 to block CB2 receptor;

Animals of inflamed group were euthanized and the bladder was harvested for the determination of AEA and PEA by mass spectrometry. This same procedure was used in control and inflamed group that received 0.7 and 7 mg/kg URB 937. The AEA and PEA values are presented as nmol/g bladder dry weight. All results are presented as mean  $\pm$  SEM.

### Results

Control group had  $0.50 \pm 0.05$  voiding contractions per minute and this was not changed by URB 937 treatment at any dose.

In LPS group, rats reflex voiding contractions at baseline were  $2.07 \pm 0.57$  contractions/ minute ( $p < 0.001$  compared to control). The administration of 0.007 mg/kg URB 937 decreased voiding contractions to  $1.70 \pm 0.37$  per minute ( $P < 0.01$  compared to control). URB 937 at 0.07 mg/kg decreased voiding contractions to  $1.20 \pm 0.21$  per minute ( $p < 0.05$  compared to control+0.07 mg URB /kg). At 0.7 mg/kg URB 937 completely reversed LPS-induced increase in voiding contractions ( $0.70 \pm 0.08$  contractions per minute,  $p > 0.05$  compared to controls). At doses of 7 mg URB /kg, the frequency of bladder reflex contractions increased (rather than decreased) to  $1.97 \pm 0.29$  contractions/minute ( $P > 0.01$  compared to control). This effect was abolished by the co-administration of 1.4 microg /kg SB366791. The administration of 10 microM CB1 antagonist MJ15 and 0.3 mg/kg CB2 antagonist SR144528 did not change the frequency of voiding contractions of naïve animals (data not shown). The co-administration of 10 microM MJ 15 + 0.7 mg/kg URB 937 induced  $0.57 \pm 0.07$  contractions per minute in the saline treated animals ( $p > 0.05$ , compared to control) and  $1.87 \pm 0.29$  contractions per minute in the LPS animals ( $p < 0.001$ , compared to control).

The co-administration of SR144528 0.3 mg/kg + 0.7 mg/kg URB 937 induced  $0.43 \pm 0.04$  contractions per minute in the saline animals ( $p > 0.05$ , compared to control) and  $0.57 \pm 0.02$  contractions per minute in the LPS animals ( $p > 0.05$ , compared to control).

The AEA and PEA levels found in the urinary bladder were the following:

	AEA (nmol/g bladder dry weight)	PEA (nmol/g bladder dry weight)
Control	$179 \pm 76$	$160 \pm 54$
LPS	$743 \pm 227$ ( $P < 0.05$ compared to control)	$4 \pm 1$ ( $P < 0.01$ compared to control)
LPS + 0.7 mg URB /kg	$190 \pm 55$ ( $P > 0.05$ compared to control)	$249 \pm 75$ ( $P > 0.05$ compared to control)
LPS + 7 mg URB /kg	$1010 \pm 470$ ( $P < 0.05$ compared to control)	$5 \pm 2$ ( $P < 0.05$ compared to control)

### Interpretation of results

URB 937 reversed bladder reflex overactivity induced by cystitis through a CB1 dependent mechanism according to a dose dependent FAAH inhibition. However at very high doses URB 937 acts on the opposite way leading to an increase in bladder reflex activity through a TRPV1 dependent mechanism.

FAAH inhibition acts by changing the levels of, at least, two different fatty acid amides.

A slight inhibition of FAAH increases the anti-inflammatory PEA and brings AEA to levels through which this endocannabinoid acts on CB1, but not on TRPV1. These two changes concur in the same direction decreasing bladder activity.

At very high doses URB 937 produced a reverse effect on AEA levels while keeping PEA levels low, as seen in cystitis. High levels of AEA had a marked stimulating effect on TRPV1 since this effect was counteracted by the TRPV1 antagonist. .

### Concluding message

This study shows that the hypothesis paradigm usually used to explain the effect of FAAH inhibitor is more complex than previously thought. The dose-dependent effects of FAAH inhibition on AEA and PEA associated with a potential inverse of the effect at high doses call for the determination of these lipids when FAAH compounds are to be used in the clinic.

### References

1. Cell Mol Life Sci. 2003 Mar;60(3):607-16

### Disclosures

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