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Disinhibition of 5-HT1A receptor control by selective 5-HT 1A receptor antagonist WAY-100635: "pharmacological model" to mimic chronic SSRI effects?

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Abstract

In previous experiments we have been observed that:

a) the cyclooxygenase-2 (COX-2) inhibitor 641784 10mg/kg or the selective serotonin reuptake inhibitor (SSRI) paroxetine 5mg/kg reduces cell firing in the Dorsal Raphe Nucleus (RDN) and that their combination shows a larger effect on this parameter.

b) 641784 10 mg/kg but not paroxetine 5 is reducing serotonin (5-HT) release in cerebral medial prefrontal cortex (mPFC), and their association does not change the COX-2 inhibitor distinct effect.

In the present work the pre-treatment with the selective 5-HT 1A receptor antagonist WAY-100635 30min before 641784 10mg/kg abolishes the reduction of 5-HT release in mPFC observed following the treatment with the COX-2 inhibitor alone. Additionally, the pre-treatment with the selective 5-HT 1A receptor antagonist WAY-100635 30min before paroxetine (5mg/kg) tended to reverse the effect of paroxetine alone on cell firing in RDN.

This data may suggest that disinhibition of 5-HT1A receptor control using selective 5-HT 1A receptor antagonist i.e. WAY-100635 could be applied as "pharmacological model" to mimic a chronic SSRI effect.

Key words: COX-2 inhibition; SSRI; In vivo voltammetry and electrophysiology; rat brain

Introduction

Published data on depressed patients suggest that the combination of cyclooxygenase-2 (COX-2) inhibition would improve the clinical outcome of the specific serotonin inhibitor (SSRI) paroxetine by alleviating depression symptoms of patients resistant to classical treatment [1-5].

In order to explore the mechanism by which this may happen we have measured the firing rate of brain neurons lying in the Dorsal Raphe Nucleus (DRN) concomitantly with the release of serotonin (5-HT) in the DRN projection structure namely the medial prefrontal cortex (mPFC) in rats injected with the COX-2 inhibitor 641784 [6], the SSRI paroxetine or a combination of both.

In previous experiments we used paroxetine at the dose 5mg/kg (s.c.) and 641784 at the dose 10mg/kg (s.c.). Both drugs induced a reduction in the cell firing of DRN neurons as well as a reduction of 5-HT release in mPFC. The combination of both drugs seemed to enhance these effects [7].

The present series of experiments have been performed to study the effect of pre-treatment with the selective 5-ht 1a receptor antagonist WAY-100635 [8, 9], upon the influence of paroxetine or 641784 alone on the electrophysiological and electrochemical parameters analysed as well as upon the association 641784 10 mg/kg + paroxetine 5mg/kg.

Methods

The methodology of associated voltammetry and electrophysiology in vivo has been described earlier [10-15].

Briefly, voltammetry associated with electrophysiology were performed at micro-biosensors stereotaxically implanted in the relevant brain areas so that neurotransmitter release and neuronal firing can be measured simultaneously in vivo, in situ, in real time before and after acute treatment in anaesthetised rats. In particular, the present experiments have been performed with microsensors implanted in RDN to monitor cell firing and in mPFC for concomitant amperometric measurement of 5-HT release.

Animals

Experiments on animals have been performed on Male Sprague-Dawley CD rats (250 g, Charles River, Italy). The animals were housed four per cage, fed with Purina Cow with water available ad libitum and kept in a temperature-controlled environment.

The experimental procedures were in line with the NIH Guidelines for Small Animal Research and approved by local review committees.

Study Design

Each rat was anaesthetized using urethane (1.4 g/kg i.p.), placed on a stereotaxic apparatus (D. Kopf, USA). For following stereotaxic preparation, micro-sensors preparation and their insertion in relevant brain regions under stereomicroscopy see ref [9-14]. Successively, concomitant amperometric and electrophysiology measurement (continuous real time analysis) were performed as described [10-15], and after 30min of control/control measurements, treatments are performed with i.e. vehicle (control animals) or relevant compound(s), minimum number of animals n=4 each group. The number of animals has been decided based upon the 3Rs, i.e. Reduction in the number of animals required; Refinement of the methodologies of analysis; Respect of the animals i.e. reducing their suffering as described [16].

Control groups were receiving the same number of injections (of vehicle) as the "treated" ones.

The effect of 641784 10mg/kg s.c alone on amperometric and electrophysiological signals have been investigated. Then those of 641784 Successively, other animals were pre-treated with the selective 5-HT 1A receptor antagonist WAY-100635 0.3mg/kg (WAY) and 30 min later injection of paroxetine alone (n=4) or the association paroxetine + 641784 (n=4) were performed.

Sample type:

1) amperometric current levels of extracellular 5-HT measured in nanoAmperes (nA)

2) electrophysiological spikes/sec as indication of cell firing.

Measure:

Real time concomitant detection of amperometric changes of basal levels of extracellular 5-HT at terminal levels (mPFC) and electrophysiological cell firing at RDN cell bodies level.

Structure: DRN: AP -7.8, ML 0, DV 6.5; mPFC: AP 3.2, L 0.5; V 4.6 (Paxinos 1986)

Results expression:

% of control; Statistical analysis details: 2ways ANOVA and Dunnett.

Results

Electrophysiological data

A) **Figure 1 left** shows that paroxetine alone is significantly decreasing RDN cell firing to approximately 53% of control values within 25-30 min. When pre-treatment 30min earlier with WAY is performed, the subsequent paroxetine treatment is no longer decreasing RDN cell firing. The recorded signal tends towards an increase that is not statistically significant due to high variability of recordings in the six animals treated (**figure 1 right**). However, statistically significant differences are observed when comparing paroxetine treatment alone versus WAY + paroxetine treatment (**figure 1 bottom**).

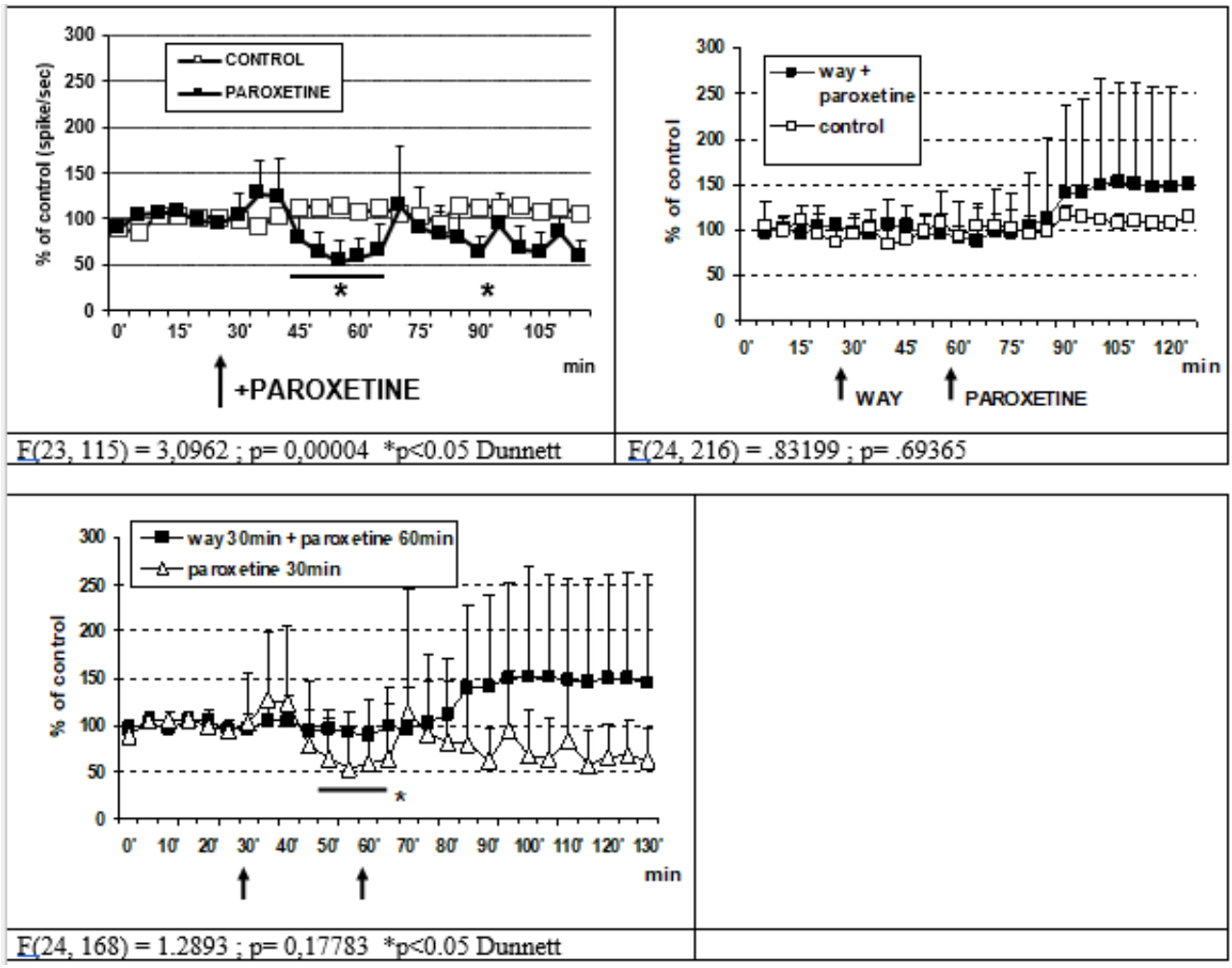


Figure 1: Electrophysiology: in vivo cell firing in RDN.

Treatments (ARROW): vehicle (CONTROL: saline 600µl s.c. n=4) versus:

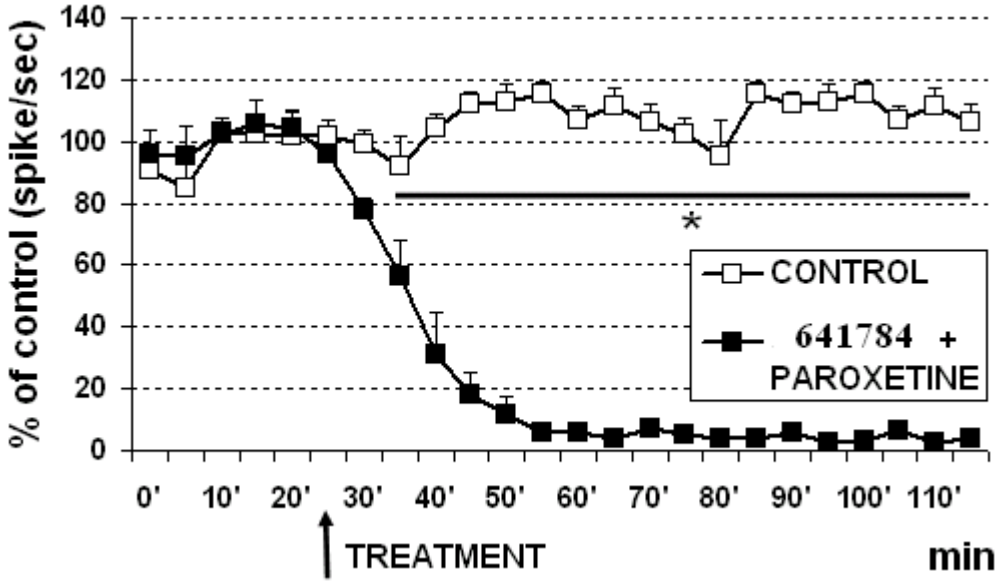
LEFT: PAROXETINE 5mg/kg (n=4 ± S.D.) OR

RIGHT: WAY 0.3mg/kg + PAROXETINE 5mg/kg s.c. n=4 ± S.D.

Stats: 2W ANOVA, Dunnett.

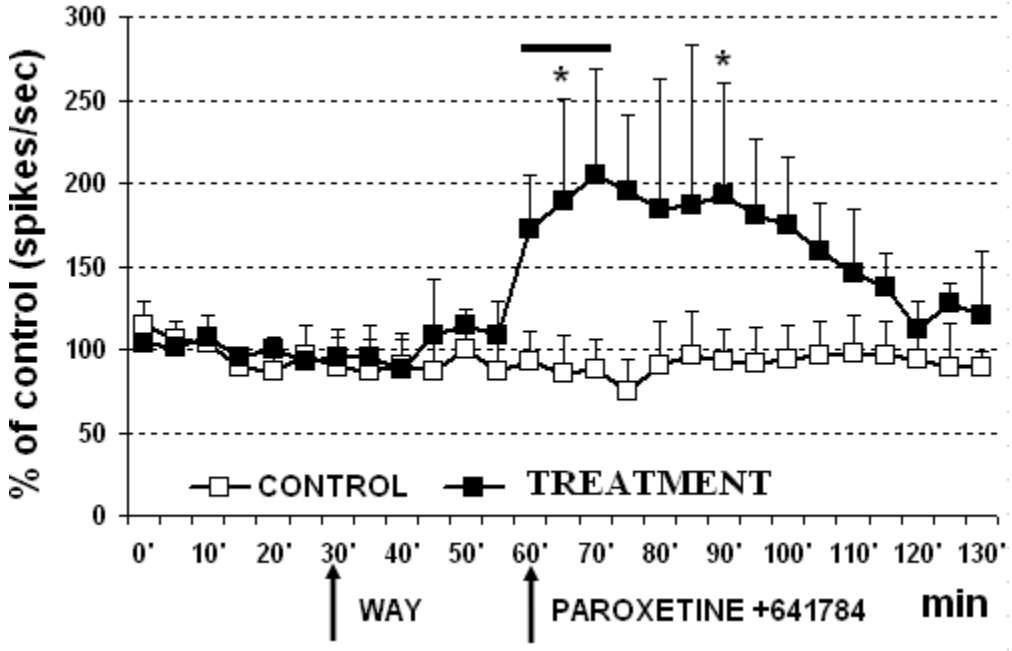
B) In the left side of **Figure 2A top** are presented the previous results obtained with the association 641784 10mg/kg + paroxetine 5mg/kg s.c. alone, showing a significant decrease of cell firing in RDN to approximately 3% of controls within 25-30min.

In a further group of rodents, the pre-treatment 30min earlier with WAY appears to significantly reverse the effect of the association 641784 10mg/kg + paroxetine 5mg/kg; as indeed RDN cell firing is increased to 200% of controls within the successive15-20min (see **figure 2A bottom**).



STATS:

F (23,138) = 36,191; p=0,0001 *p<0.05 Dunnett



STATS: F (26,156) = 4,8179; p=0,000001 *p<0.05 Dunnett

Figure 2A: ELECTROPHYSIOLOGY: in vivo cell firing in RDN.

Treatments (ARROW): vehicle (CONTROL: saline 600µl s.c.) versus:

TOP: 641784 10mg/kg + PAROXETINE 5mg/kg OR

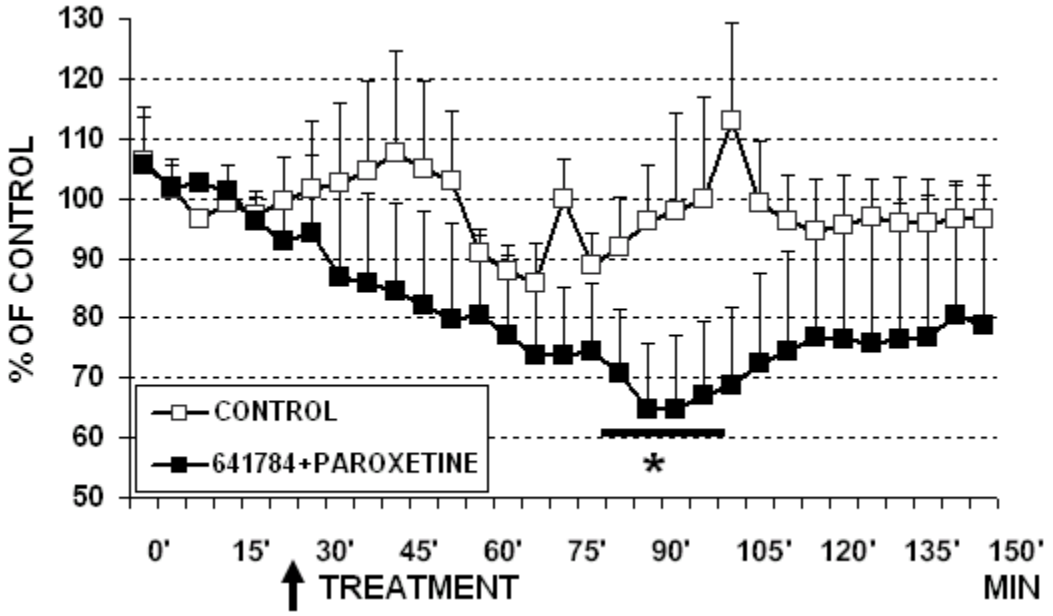
BOTTOM: WAY 0.3mg followed by 641784 10mg + PAROXETINE 5mg/kg.

n=4 ± S.D. each group; Stats: 2W ANOVA, Dunnett.

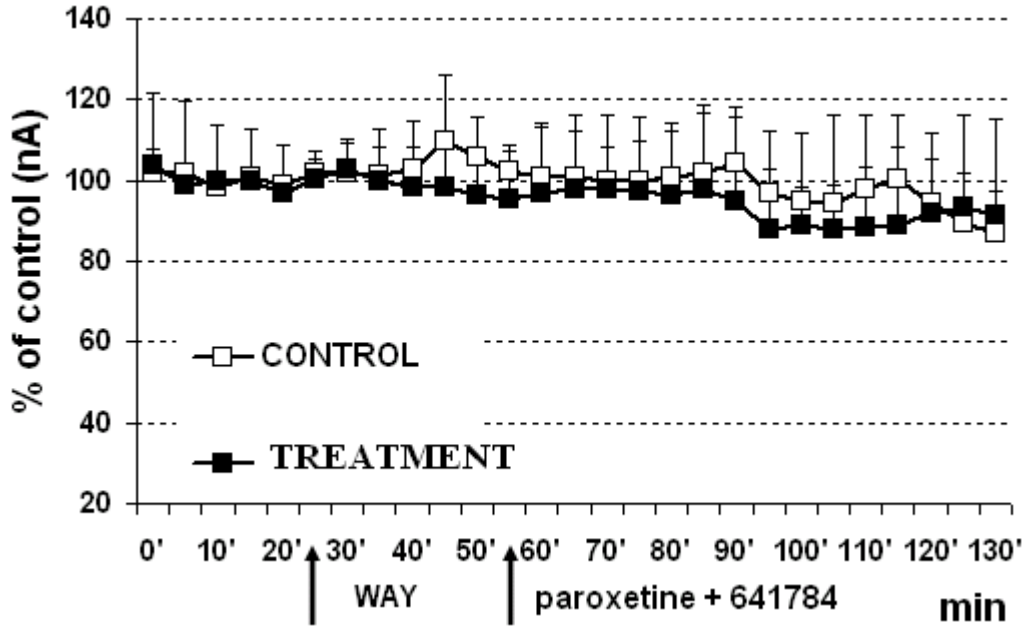
Amperometric data

The analysis of the amperometric 5-HT release in prefrontal cortex shows that while the association paroxetine 5mg + 641784 10mg is significantly decreasing 5-HT release to approximately 64% of control values within

60min (**figure 2B top**), pre-treatment 30 min earlier in a further group of rodents with WAY reverses such effect as 5-HT levels are in that case not significantly different than control values (**figure 2B bottom**).



STATS: F (23,138) = 36,191; p=0,0001 *p<0.05 Dunnett



STATS: F (26,182) = 0,33314; p=0,99919

Figure 2B: Concomitant AMPEROMETRY: 5-HT levels in prefrontal Cortex.

Treatments (ARROW): vehicle (CONTROL: saline 600µl s.c.) versus:

TOP: 641784 10mg/kg + PAROXETINE 5mg/kg OR

BOTTOM: WAY 0.3mg followed by 641784 10mg + PAROXETINE 5mg/kg.

n=4 ± S.D. each group; Stats: 2W ANOVA, Dunnett.

Discussion

Efficacy of SSRIs upon major depression, anxiety and other CNS illness has been confirmed [17, 18, 19]. However, a significant positive action of such treatment requires a delay that varies from as little as a week until after several weeks of treatment [20, 21, 22, 23, for a review see 24].

The present experiments show that:

i) pre-treatment with WAY is reversing the effect of paroxetine on RDN cell firing indicating that such approach could be mimicking the state of a chronic SSRI treatment. Therefore, this approach could be applied as "acute pharmacological therapeutic model" of disinhibition of 5-HT1A receptor control on RDN activities so that to study compounds efficacy within a virtual state of chronic SSRI effect. This is supported by the evidence that pre-treatment with WAY also reverse the reduction of 5-HT release monitored by amperometry in prefrontal cortex following the association

641784 10mg/kg + paroxetine 5mg/kg.

ii) the electrophysiological data that show that WAY pre-treatment followed by such association is resulting in significant increase of RDN cell firing.

This data may suggest that disinhibition of 5-HT1A receptor control using selective 5-HT 1A receptor antagonist i.e. WAY-100635 could be applied as "pharmacological model" to mimic a chronic SSRI effect.

Further work to verify this possibility will be performed in animal model of depression [25, 26] also prepared for wireless electrochemical-electrophysiological monitoring in conscious animals [10, 14, 27, 28]. Treatments with SSRIs alone or preceded with WAY in such animals will be performed to test this hypothesis.

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