

# Microdialysis in vivo evaluation of the effects of SA-4503, a sigma1 receptor agonist, on the levels of monoamines in the prefrontal cortex of conscious rats

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**Received date:** February 26, 2021; **Accepted date:** March 30, 2021; **Published date:** April 06, 2021

**Citation:** Francesco Crespi (2021) Microdialysis in vivo evaluation of the effects of SA-4503, a sigma1 receptor agonist, on the levels of monoamines in the prefrontal cortex of conscious rats. *J. Neuroscience and Neurological Surgery*. 8(4); DOI:10.31579/2578-8868/164

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## Abstract:

Sigma receptors are widespread in the central nervous system and are differentiated in two subtypes, sigma1 and sigma2. In particular, the sigma1 receptor subtype appears to be able to influence biological mechanisms connected with neuro-degeneration. Furthermore, several studies are implicating sigma1 receptor agonists within antidepressant activity.

Evidence of prefrontal cortex abnormalities in clinically depressed subjects have been reported by several works and that monoamines such as serotonin and catecholamines can be involved in such malfunctions.

Up to now the most of the preclinical work performed to analyze the influence of sigma1 receptor agonists upon catecholaminergic and serotonergic activities in brain areas has been done by means of in vitro as well as ex vivo methodologies.

Here, SA-4503, a selective sigma1 receptor agonist with potential antidepressant activity has been tested in vivo upon dopamine (DA), noradrenaline (NA) and serotonin (5-HT) levels detected by micro-dialysis in the medial Prefrontal Cortex (mPFC) of freely moving rats.

**Keywords:** sigma1 receptor; SA-4503; medial Prefrontal Cortex, freely moving rats; micro-dialysis; dopamine, noradrenaline and serotonin

## INTRODUCTION

Sigma receptors are widespread in the central nervous system and across multiple peripheral tissues. Sigma receptors were initially described in 1976 as opiate receptors. They were successively differentiated in two subtypes, sigma1 and sigma2 [1, 2]. More recently it has been shown that these receptors are non-opioid, are trans-membrane proteins and play various purposes in intracellular signaling, apoptosis and metabolic regulation (for a review see ref. 3). Besides, several studies propose that Sigma receptors undergo various functions in psychiatric diseases. It has been shown that sigma receptors and in particular the sigma1 receptor subtype is expressed in both neuronal as well as cerebral glia cells and appear to be able to influence biological mechanisms connected with neuro-degeneration [4] Thus, sigma1 receptors may be considered as targets for the development of pharmacological approaches to treat various CNS disorders (for a review see ref. 5).

In particular accrued indication are proposing a function for these sigma receptors in antidepressant effects [6, 7].

Cerebral catecholamines as well as serotonin have been implicated in psychiatric diseases induced either by defined genetic etiologies and pathological phenotypes that result in neurodegenerative disorders (for a review see refs 8, 9, 10).

For what concerns catecholamines, various experiments, mainly performed in vitro as well as ex vivo, have shown that sigma1 receptors are one of the endogenous substrates that are able to antagonize cytotoxicity connected to dopamine activity [11], toxicity that is responsible of apoptosis and therefore can be leading to Parkinson disease [12] i.e. endogenous dopamine can undergo both enzymatic and auto-oxidation, generating ROS and causing degenerative damage to dopaminergic neurons [13].

Disorder in central noradrenergic transmission in depression has been described [14, 15]. Mainly a complex alteration of noradrenergic function is observed, most consistent with over-activation of this system (for a review see ref. 16).

The involvement of serotonin in the pathophysiology of depression is suggested by various observations [17, 18] and various studies have shown that long-lasting treatments with antidepressants increase 5-HT neurotransmission [19, 20].

Magnetic resonance imaging (MRI) and computed tomography (CT) as well as positron emission tomography (PET) and single photon emission computed tomography (SPECT) i.e. structural and functional imaging

have indicated that the prefrontal cortex, is being directly involved in clinical depression. Indeed these studies have shown evidence of prefrontal cortex abnormalities in clinically depressed subjects i.e. functional imaging studies demonstrate prefrontal lobe hypo-metabolism in primary and secondary depression, with severity of depression often correlating with the degree of frontal inactivity [21, 22, 23].

Up to now the most of the preclinical work performed to analyze the influence of sigma1 receptor agonists upon catecholaminergic and serotonergic activities in brain areas has been done by means of in vitro as well as ex vivo methodologies. In vivo studies have been also performed but mainly by means of electrophysiologic analysis of cell firing in discrete brain areas, with no biochemical evidence of specific neurotransmitter(s) changes [24].

In the present work, the effects of SA-4503, a selective sigma1 receptor agonist with potential antidepressant activity [24, 25] has been tested in vivo upon dopamine (DA), noradrenaline (NA) and serotonin (5-HT) levels in the medial Prefrontal Cortex (mPFC) of freely moving rats prepared for micro-dialysis as described earlier [26, 27, 28].

The choice of such selective sigma1 receptor agonist was set by the evidence of a high specific uptake of [ $^{11}\text{C}$ ] SA4503 in the brain frontal cortex in *ex vivo* autoradiography (ARG) and PET studies [29, 30].

## METHODS

A microdialysis implant was applied as described earlier [26, 27] to four groups of male rats (n=10 each group) that were randomly tested with the vehicle (aCSF, 2ml/kg p.o.) and the sigma1 agonist at doses 1, 3 and 10 mg/kg. Before (basal levels) and following drug treatments microdialysis samples were collected every 20 minutes during 3 hours.

### Animals

Male adult rats (Wistars, 250-280 g) were supplied by Charles-River (Italy) and kept in temperature- and humidity-controlled rooms (22 °C, 50%). All animal procedures were carried out in accordance with the Italian law (Legislative Decree no. 116, 1992) which acknowledges the European Directive 86/609/EEC. In addition, all efforts were made to minimize the number of animals and their suffering accordingly with ref. 31.

The animals were anaesthetized (urethane, 2 g/kg i.p.) and then prepared for brain microdialysis in the mPFC (stereotaxic coordinates from ref. 32) as already described [26, 27]. The treatment with SA-4503 (provided by M's Science Corporation, Kobe, Japan) was performed accordingly with ref. 33. In particular the doses 1, 3 and 10mg/Kg p.o. were selected for single administration of this sigma1 receptor agonist.

### Micro-dialysis

Brain microdialysis is based upon the sampling of cerebral extracellular fluid by means of a cannula built and then surgically implanted into the tissue to be studied as described earlier [26, 27]. Briefly, fiber dialysis membrane 0.2–0.3 mm in diameter are inserted into a metal cannula and perfused with an iso-osmolar physiological fluid such as artificial cerebral spinal fluid (aCSF).

The outflow dialysate contains the molecules that traverse into the aCSF fluid because of the concentration gradient between the perfusate and the extracellular space. It is collected every 20 minutes and then basal levels of DA, NA and 5-HT in each dialysate are measured by using an HPLC system with electrochemical detection for separation and quantification as described earlier [26, 27].

Formulation of artificial cerebrospinal fluid (aCSF) in mM: 125 NaCl, 2.5 KCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 25 NaHCO<sub>3</sub>, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 25 glucose bubbled with 95% O<sub>2</sub>, 5% CO<sub>2</sub>, then add 2 CaCl<sub>2</sub>. Filter with a 0.22- $\mu\text{m}$  filter apparatus, and store at 4°C.

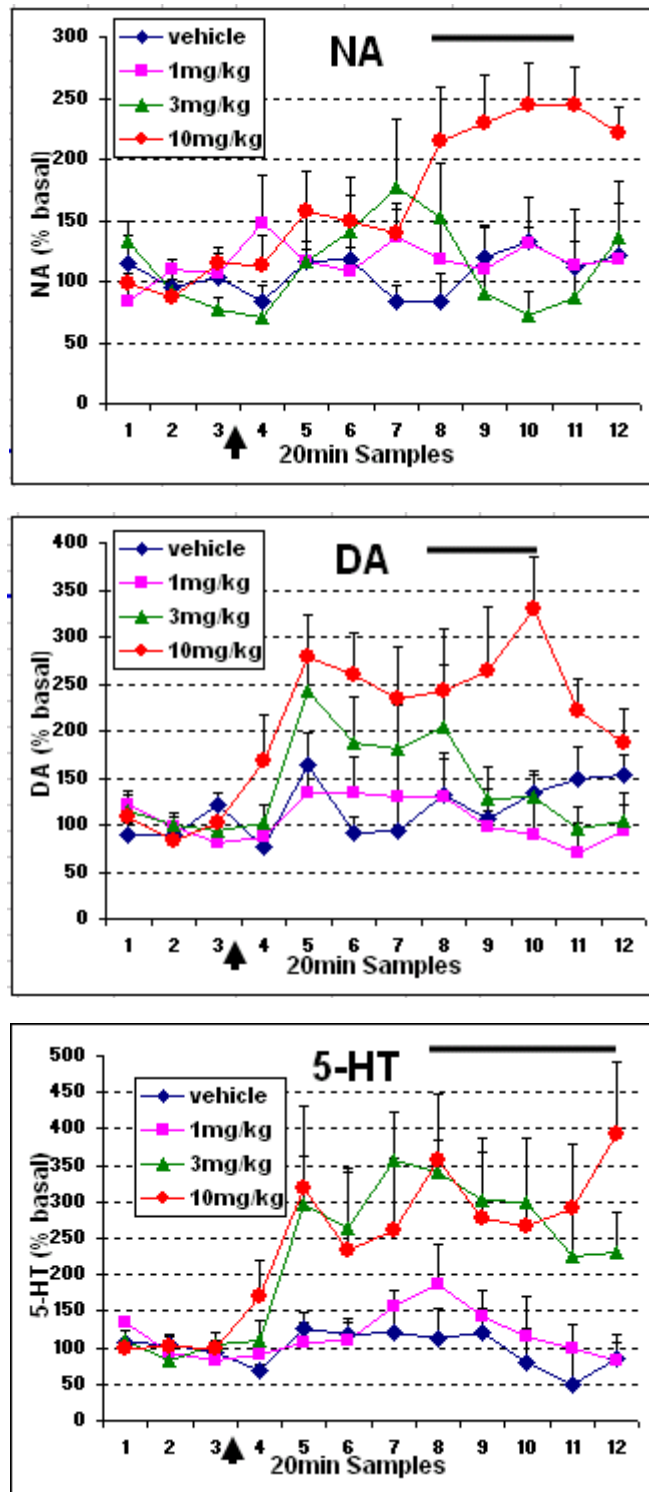
### Data analysis

The data obtained from all the experiments were converted to percent from basal values and analyzed with STATISTICA software version 6.0 using repeated-measures ANOVA, with treatment and time as main factors. The Fisher LSD test was used as post hoc to evaluate significant differences between mean values produced by drug treatments versus controls (vehicle treatment) at each time point. Statistical significance was set at a probability level of  $p < 0.05$ .

## RESULTS

The in vivo data collected in the mPFC following the treatment with SA-4503 showed that:

- the dose 1mg/kg was unable to modify significantly any of the three signals monitored
- the doses 3mg/kg and 10mg/kg appeared to be able to modify DA in a rather dose – dependent manner and 5-HT levels in a similar manner (see figure 1 middle and bottom). In particular, concerning NA, the highest dose was able to increase significantly the related electrochemical signal up to approx. 200% over the control values within 80-100min (figure 1 top). The values of DA increased up to 300% of controls following the middle and highest dose within 20-30min and up to approx. 350% of controls within 80-100min (figure 1 middle). The values of 5-HT increased up to approx. 300% of controls following 3 and 10mg/Kg SA-4503 within 20-30min and up to approx. 350% within 80-90min. Successively, the effect of the 3mg/kg dose tended to reduce while the highest dose was determining a further increase of 5-HT levels up to approx. 400% of controls within 180min (figure 1 bottom).



**Figure 1** shows the effect of vehicle (aCSF 2ml/kg p.o., n=10) and that of the sigma1 receptor agonist SA-4503 at doses 1, 3 and 10 mg/kg (n=10 each dosage) upon extracellular levels of NA, DA and 5-HT collected within the mPFC of conscious rats.

Before (basal levels) and following each treatment (arrow) microdialysis samples were collected every 20 minutes during 3 hours. In each panel, black solid line indicates statistical significance set at a probability level of  $p < 0.05$ .

## DISCUSSION

The present in vivo experiments performed in conscious animals have

shown that treatment with the sigma1 receptor agonist SA-4503 is followed by increase of the three monoamines monitored in the mPFC. In

particular, the dose 3 and 10mg/Kg resulted in significant increase of the electrochemical signal related to 5-HT while the dosage of 10mg/Kg in that of NA and DA. This is in accord with previous work showing a maximum effect of 10mg/Kg dose of SA-4503 upon electrophysiological, morphological and behavioural parameters monitored in anaesthetized as well as behaving rodents [33]. Furthermore, the present data is qualitatively in line with some literature reports about the putative capability of sigma1 receptor agonist to facilitate DA release in prefrontal cortex [34, 35].

In addition, evidence for an improved extracellular level of NA in this area further support the implication of catecholamines within the sigma1 receptor agonists influence in such brain region, and in particular their positive effect in behavioural models of depression such as the tail suspension [36, 37] and the forced swimming test [38, 39].

Taken together with the electrophysiological, morphological and behavioural observations reported in ref. 33 in which it appears that functional changes following SA-4503 treatment occurred within a short time-frame (2–3 days) all these data provide both functional and behavioural evidence that this compound has an important antidepressant potential; moreover they suggest that this antidepressant potential might have a rapid onset of action.

Concerning serotonin, several works suggest that sigma1 receptors are involved in the active mechanisms of selective serotonin reuptake inhibitors (SSRIs) so that sigma1 receptor agonists are proposed as potential therapeutic drugs for the treatment of cognitive impairment in schizophrenia, psychotic depressions well as neurodegenerative disorders (for a review see refs 40, 41). Furthermore, it has been shown that sigma ligands are able to speedily produce an increase in 5-HT firing activity recorded in the dorsal raphe nucleus (DRN) with a more rapid and robust effect than the vast majority of known antidepressant medications [3, 42].

The interaction of sigma receptors and neurotransmitters is complex, and although a lot of work has been done in this field, most work is done in vitro (for a review see ref. 3). In the attempt to help in elucidating such interaction, the present observations are proposing a rapid significant positive influence of SA-4503 upon neuroamines monitored in vivo in the pFC, and in particular on those involved in psychiatric diseases [8, 9, 10], thus strengthening the argument for sigma receptor's role in depression and proposing sigma ligands as potential antidepressant with a rapid onset of action.

#### Acknowledgement to:

Francesco Congestri, Chiara Lazzarini for technical support

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DOI:[10.31579/2578-8868/164](https://doi.org/10.31579/2578-8868/164)

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