Received: 24 November 2020 | Revised: 22 April 2021 | Accepted: 14 May 2021

DOI: 10.1111/ejn.15319

SHORT COMMUNICATION

EJN European Journal of Neuroscience FENS WILEY

Participation of glutamatergic and nitrergic systems in the striatal dopamine release induced by isatin, a MAO inhibitor

Lilian R. F. Faro | **Lorenzo Justo** | **Raquel Gómez** | **Rafael Durán**

Department of Functional Biology and Health Sciences, University of Vigo, Vigo, Spain

Correspondence
Lilian R. F. Faro, Department of Functional Biology and Health Sciences, University of Vigo, Campus Lagoas-Marcosende, 36310 Vigo, Spain.
Email: lilianfaro@uvigo.es

Edited by: Serge Schiffmann

Abstract

Isatin is a biofactor with different biochemical and pharmacological properties whose effects attract much attention because it is an endogenous inhibitor of the monoamine oxidase in the brain. When exogenously administrated, isatin increases dopamine levels in intact and denervated striatum of rats, an effect that could indicate its potential as a therapeutic agent in Parkinson disease. However, the neurochemical mechanisms by which isatin increases dopamine in the striatum are poorly understood. In the present study, we evaluate the role of the glutamatergic and nitrergic systems in the isatin-induced dopamine release from rat striatum. Our findings show that the intrastriatal administration of 10 mM isatin significantly increases the in vivo release of dopamine (1,104.7% ± 97.1%), and the amino acids glutamate (428.7% ± 127%) and taurine (221% ± 22%) from rat striatum measured by brain microdialysis. The pretreatment with MK-801 (500 µM) or AP5 (650 µM) (glutamatergic NMDA receptors antagonists) significantly reduces the effect of isatin on dopamine release by 52% and 70.5%, respectively. The administration of the nitric oxide synthase inhibitors, L-NAME (100 µM) or 7-NI (100 µM) also decreases the isatin-induced dopamine release by 77% and 42%, respectively. These results show that isatin, in addition to increasing dopamine release, also increases glutamate levels, and possibly activates NMDA receptors and nitric oxide production, which can promote a further increase in the dopamine release.

KEYWORDS

brain microdialysis, glutamate, in vivo dopamine release, isatin, rat striatum

Abbreviations: 7-NI, 7-nitroindazole; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ANOVA, analysis of variance; ANP, atrial natriuretic peptide; AP5, (2R)-amino-5-phosphonovaleric acid (AP5); BNP, brain natriuretic peptide; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; HPLC-ED, High-Performance Liquid Chromatography with electrochemical detection; HPLC-FD, High-Performance Liquid Chromatography with fluorescent detection; HVA, homovanillic acid; ISA, isatin; L-NAME, $N^{\dot{\omega}}$-Nitro-L-arginine; MAO, monoamine oxidase; MK-801, dizocilpine; NMDARs, N-methyl-D-aspartate receptors; NO, nitric oxide; NOS, nitric oxide synthase; PD, Parkinson disease; VDCC, voltage-dependent calcium channels.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2021 The Authors. European Journal of Neuroscience published by Federation of European Neuroscience Societies and John Wiley & Sons Ltd

1 | INTRODUCTION

Isatin (indole-2, 3-dione) is an organic compound derived from indole that has different biochemical and pharmacological properties (Hamaue et al., 1999; Medvedev et al., 1996). It represents an important class of compounds that can be used as precursors for drug synthesis and a lot of research work has been done regarding its synthesis, chemical properties, biological and industrial applications.

Isatin presents a wide spectrum of biological properties such as a marker of stress and anxiety, as an inhibitor of a number of enzymes, as an anti-convulsant agent, and as an inhibitor of benzodiazepine, of atrial natriuretic peptide (ANP) and of brain natriuretic peptide (BNP) receptors (Medvedev et al., 2018). We understand that this substance can present multiple mechanisms of action and the elucidation of such mechanisms responsible for the isatin effects requires identification of particular targets, which would demonstrate the possible benefits of this molecule as neuroprotector, anxiolytic, antioxidant, etc.

The effects and mechanisms of isatin's action in the brain attract much attention since isatin is an endogenous inhibitor of the monoamine oxidase (MAO) (Hamaue et al., 1999; Manley-King et al., 2011; reviewed by Medvedev et al., 2018). MAO inhibitors have great clinical importance and are used as adjunct therapeutic agents in Parkinson disease (PD). Isatin is found in several tissues in the μM range, but in stressful situations, this concentration significantly increases, but the mechanism responsible for this increase is unknown (Medvedev et al., 2005, 2018; Medvedev & Glover, 2004, for review). The administration of isatin has been shown to increase monoamines and acetylcholine levels in the intact rat brain (Minami et al., 2006). Moreover, in rat models of PD, the isatin treatment prevents dopamine depletion in the striatum (Hamaue et al., 2004; Ogata et al., 2003). However, another research seems to contradict these results, for it indicates that systematically administered isatin did not increase the striatal dopamine levels in parkinsonized rats, although it improves motor signs (Zhou et al., 2001).

Isatin can also act as an inductor of exocytotic dopamine release. A study from our laboratory showed that intrastriatal infusion of isatin increases the levels of striatal dopamine in a concentration-dependent manner, being this increase dependent on the depolarization of the dopaminergic terminal as well as dependent on the presence of Ca^{++} in the extracellular medium, and also dependent on the storage of dopamine in the synaptic vesicles (Justo et al., 2016). Furthermore, we have recently proven that the administration of isatin together with antiparkinsonian drugs significantly improves the extracellular levels of striatal dopamine, showing that, in addition to its effect as a MAO inhibitor, it can also act as a dopamine releaser (Faro et al., 2020).

In the present work, we have extended our in vivo studies to investigate the involvement of N-methyl-D-aspartate receptors (NMDARs) and nitric oxide (NO) production in the effects of isatin on striatal dopamine release. To achieve this purpose, we have used (2R)-amino-5-phosphonovaleric acid (AP5), a competitive antagonist of NMDARs, dizocilpine (MK-801), a non-competitive antagonist of NMDARs, 7-nitroindazole (7-NI) a selective inhibitor of nitric oxide synthase (NOS), and $N^{\dot{\omega}}$-Nitro-L-arginine (L-NAME), a NOS inhibitor. We have also investigated the effect of isatin on the striatal levels of some amino acids with neurochemical signification, mainly the glutamate, in order to complete the possible mediation of the glutamatergic system on the neurochemical effects of isatin.

2 | MATERIALS AND METHODS

Adult female Sprague-Dawley rats were used ($n = 45$; 250–300 g; Breeding Facility of the University of Santiago de Compostela). Animals were housed in plastic cages (22 cm high, 45 cm deep, and 23 cm wide), kept in a humidity-controlled colony room with a constant temperature (22 ± 2℃) and a 14/10 hr light/dark cycle, with free access to water and standard laboratory chow. Animals were treated in accordance with the Guidelines of the European Union (2010/63/EU) and the Spanish Government (*Real Decreto* 53/2013) for the use of animals for experimental purposes. This study was approved by the Ethics Committee on Animal Welfare of the University of Vigo. All possible efforts were made to minimize animal suffering and reduce the number of animals used.

In all the experiments, analytical grade reagents were used, and the water was previously purified with a Milli Q system (Millipore). Isatin, 7- NI, L-NAME, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), aspartate, glycine, glutamate, taurine and chloral hydrate, were provided by Sigma-Aldrich. AP5 and MK-801 were provided by Tocris. All drugs were dissolved in Ringer solution and administered into striatum through a dialysis probe.

Drug concentrations were selected based on literature and on our previous works about the involvement of glutamatergic receptors and NO production on stimulated dopamine release measured by brain microdialysis, where it has been observed that the concentrations used were enough to block the receptors and to inhibit the enzyme and, thus, to attenuate the stimulation of in vivo dopamine release (Alfonso et al., 2003; Campos et al., 2006; Faro et al., 2002, 2013).

All experiments were made in awake, conscious and freely moving animals and were carried out for over 3 or 4 hr, as it had been done in previous studies from our laboratory (Faro et al., 2020; Justo et al., 2016). Forty-four animals were

divided into seven groups as presented in Table 1. The present study was carried out with female rats. Previous data from our laboratory did not show significant differences between males and females both in baseline dopamine levels and in the effects observed by the intrastriatal administration of different treatments (Alfonso et al., 2003; Arias et al., 1998; Faro et al., 2020; Justo et al., 2016). Furthermore, in a recent systematic review by Egenrieder et al. (2020), these authors also did not observe significant differences between males and females; neither relates to the basal levels of dopamine found in the striatum nor the effects produced by the administration of drugs of abuse.

The surgery for the guide-cannula implantation into the left striatum and microdialysis experiments were performed based on the methods described previously (Faro et al., 2020; Justo et al., 2016). When starting the microdialysis experiment, the probe was introduced into striatum and perfused with a Ringer solution (147 mM NaCl, 4 mM KCl and 2.4 mM $CaCl_2$, pH = 7.4). After the stabilization time and the collection of three basal samples (60 min), isatin was infused during 60 min. Subsequently, the medium was switched back to the Ringer solution and the sampling continued for an additional period of 60 min. For the study of the effects of glutamate antagonists and NOS inhibitors, after collecting three basal samples, the different drugs were infused for 60 min and then mixed with isatin, which was infused throughout the third hour of the experiment. The next step was switching the medium back to the Ringer solution (60 min). After every experiment, the animal was euthanized by deep anesthesia and cervical dislocation and then the brain was removed for the histological verification of the correct localization of the probe.

The dopamine and its metabolites were measured by High-Performance Liquid Chromatography (HPLC) with electrochemical detection (ED) according to previous studies from our laboratory (Faro et al., 2020; Justo et al., 2016). A Jasco PU-1580 pump and a Reodyne 7125 injector were used, and a mobile phase was eluted through a 20 cm Spherisorb ODS-1 reverse phase column (5 µm particle size). The dopamine, DOPAC and HVA detection was achieved by using an ESA Coulochem III 5100A electrochemical detector at a potential of +400 mV.

Amino acids levels were measured by HPLC with fluorescent detection (HPLC-FD) according to previous studies from our laboratory (Faro et al., 2013). Briefly, the samples of amino acids were derivatized using o-phthaldialdehyde and N-acetyl-l-cysteine. The HPLC-FD system was equipped with a Jasco PU-1580 pump, a Reodyne 7125 injector and a CMA 280 fluorescence detector. The separation of amino acids was accomplished by using Kromasil 100 C18 reversed-phase columns. Amino acids were detected using 330–365 nm (excitation) and 440–530 nm (emission).

All values of the effects of isatin, receptor antagonists or inhibitors on extracellular levels of dopamine and metabolites were expressed as mean ± *SEM* of 4–6 animals in each group. The average basal levels of dopamine, DOPAC, HVA, and amino acids (defined as 100%) were determined from the two dialysate samples before the addition of isatin or any drug. Results were calculated as percentages of this average basal release. Data of dopamine, DOPAC, HVA and amino acids were corrected using the percentage of in vitro recovery for every microdialysis probe.

The statistical significance of differences between groups for dopamine and amino acids was assessed by repeated-measures analysis of variance (ANOVA) with time (TIME) as a within-subject factor and group (GROUP) as a between-subject factor. To investigate whether the time effect differed between groups, we confirmed the “TIME” × “GROUP” interaction. Data at each time point were compared with the basal using the paired *t* test to investigate when dopamine levels became prominent. Post hoc comparisons were made using Bonferroni's test. The effects of treatments on DOPAC and HVA were analyzed by one-way ANOVA followed by Bonferroni's multiple comparison test. The accepted level of significance for the tests was $p \leq 0.05$. All tests were performed using the SPSS 220.0 (SPSS Inc.).

TABLE 1 Experimental groups in which drugs were dissolved in the perfusion fluid and administered into striatum through the dialysis probe

	Time of administration			
Groups	**60 min**	**60 min**	**60 min**	**60 min**
Dopamine, DOPAC and HVA ($n = 10$)	Ringer	Isatin 10 mM	Ringer	—
Amino acids ($n = 5$)	Ringer	Isatin 10 mM	Ringer	—
Control ($n = 4$)	Ringer	Ringer	Ringer	—
MK-801 + isatin ($n = 6$)	Ringer	500 µM MK-801	500 µM MK-801 + 10 mM isatin	Ringer
AP5 + isatin ($n = 6$)	Ringer	650 µM AP5	650 µM AP5+10 mM isatin	Ringer
L-NAME + isatin ($n = 8$)	Ringer	100 µM MK-801	100 µM L-NAME+10 mM isatin	Ringer
7-NI + isatin ($n = 5$)	Ringer	100 µM 7-NI	100 µM 7-NI+10 mM isatin	Ringer

3 | RESULTS

3.1 | Effects of isatin on striatal dopamine, DOPAC and HVA levels

In the present study, we confirmed that intrastriatal infusion of isatin increased the in vivo dopamine release from rat striatum (Justo et al., 2016). Table 2 shows the mean of the concentrations of dopamine, DOPAC and HVA in the two previously collected samples before isatin infusion, which were considered as the basal levels, and the maximal effect of the isatin on the extracellular levels of these substances. Intrastriatal administration of 10 mM isatin (60 min) increased the dopamine levels up to 1,104% ± 97.1% ($p < 0.001$, t test), compared to the baseline, 40 min after the start of isatin administration, and significantly decreased the DOPAC and HVA levels, reaching the minimum values of 70.9% ± 12.6% ($p < 0.001$, t test) and 81.4% ± 6.4% ($p < 0.01$, t test), respectively, regarding the baseline, 80 min after the start of isatin administration.

3.2 | Mediation of NMDA glutamatergic receptors in the effect of isatin on striatal dopamine release

In this group of experiments, we studied the effect of MK-801 (500 μM) or AP-5 (650 μM) pretreatment on isatin-induced dopamine release (Figure 1A). The infusion of MK-801 or AP-5 for one hour through the microdialysis probe does not significantly change the extracellular dopamine levels. The perfusion of isatin to animals pretreated with MK-801 or AP-5 produced a significant increase in dopamine levels, reaching a maximum of 534.4% ± 160.0% ($p = 0.00003$, t test) and 326.7% ± 71.8% ($p = 0.00008$, t test), compared to basal levels, 40 min after the start of isatin administration, respectively (Figure 1A). These effects on dopamine extracellular levels were significantly smaller than that produced by isatin at the same times, assuming a reduction of 52% and 70% in the maximal effect of isatin. Repeated measures ANOVA for dopamine differed significantly between the three groups analyzed: isatin, isatin + MK-801 and isatin + AP-5 [main effect of Group, $F(2,19) = 6.18$, $p = 0.009$; main effect of Time, $F(11,209) = 25.54$, $p < 0.001$; interaction of Group × Time, $F(11,209) = 3.26$, $p < 0.001$]. Multiple comparisons indicate significant differences between the effects of isatin alone and isatin + MK-801 or isatin + AP-5 ($p = 0.04$ and $p = 0.016$, respectively).

Figure 1B–C shows that administration of MK-801or AP-5 does not significantly change the levels of the metabolites. Although, AP-5 produced a slight but significant increase in HVA levels up to a maximum of 131.9% ± 4.6% ($p < 0.01$, t test) relative to basal levels. The infusion of isatin to animals pretreated with MK-801 or AP5 significantly decreased the DOPAC up to 75.6% ± 9.6% ($p = 0.0070$, t test) and 63.9% ± 10% ($p = 0.0033$, t test), respectively, compared to basal levels, 80 min after the start of administration its infusion, and does not significantly change the HVA levels. Besides, these declines did not differ significantly from the effect of isatin on both metabolites at the same time. The one-way ANOVA confirmed the existence of significant differences for the effects of MK-801 or AP-5 on DOPAC ($F(3,28) = 15.40$, $p < 0.001$ and $F(3,28) = 22.60$, $p < 0.001$, respectively) and HVA ($F(3,28) = 15.18$, $p < 0.001$ and $F(3,28) = 7.89$, $p < 0.001$, respectively). Post hoc comparisons indicate that the effects of administering isatin to animals pretreated with MK-801 or AP5 do not differ significantly from those observed with the administration of isatin alone for DOPAC ($p = 1.00$ and $p = 1.00$, respectively) and HVA ($p = 0.366$ and $p = 0.902$, respectively).

TABLE 2 Mean of basal values of dopamine, DOPAC, HVA and amino acids in all experimental groups and maximal effects of intrastriatal administration of isatin (ISA)

Substance	Basal (ng/20 min)	ISA 10 mM (ng/20 min)
Dopamine	0.30 ± 0.15	3.40 ± 1.10***
DOPAC	30.20 ± 7.00	16.60 ± 6.00**
HVA	1.90 ± 0.50	1.60 ± 0.40**
Glutamate	2.14 ± 0.50	8.40 ± 1.90***
Aspartate	1.80 ± 0.50	3.20 ± 0.10**
Taurine	0.30 ± 0.10	0.50 ± 0.20**
Glycine	0.25 ± 0.03	0.30 ± 0.03

**$p < 0.01$.; ***$p < 0.001$.

3.3 | Mediation of nitric oxide in the effect of isatin on striatal dopamine release

Figure 2A shows that the infusion of L-NAME (100 μM) or 7-NI (100 μM) for one hour, through the microdialysis probe, does not significantly change the extracellular dopamine levels. Administration of isatin to animals pretreated with L-NAME or 7-NI produced a maximal increase in dopamine levels of 360.6% ± 34% ($p = 0.0031$, t test) and 638.6% ± 159% ($p = 0.0064$, t test), compared to basal levels, 40 and 60 min after the start of isatin administration, respectively. These effects on dopamine extracellular levels were significantly smaller than that produced by isatin at the same times, assuming a reduction of 70% and 52% in the maximal effect of isatin. Repeated measures ANOVA for dopamine differed significantly between the three groups analyzed: isatin, isatin + L-NAME and isatin + 7-NI [main effect of Group, $F(2,20) = 9.01$, $p = 0.002$; main effect of Time,

EJN European Journal of Neuroscience FENS —WILEY

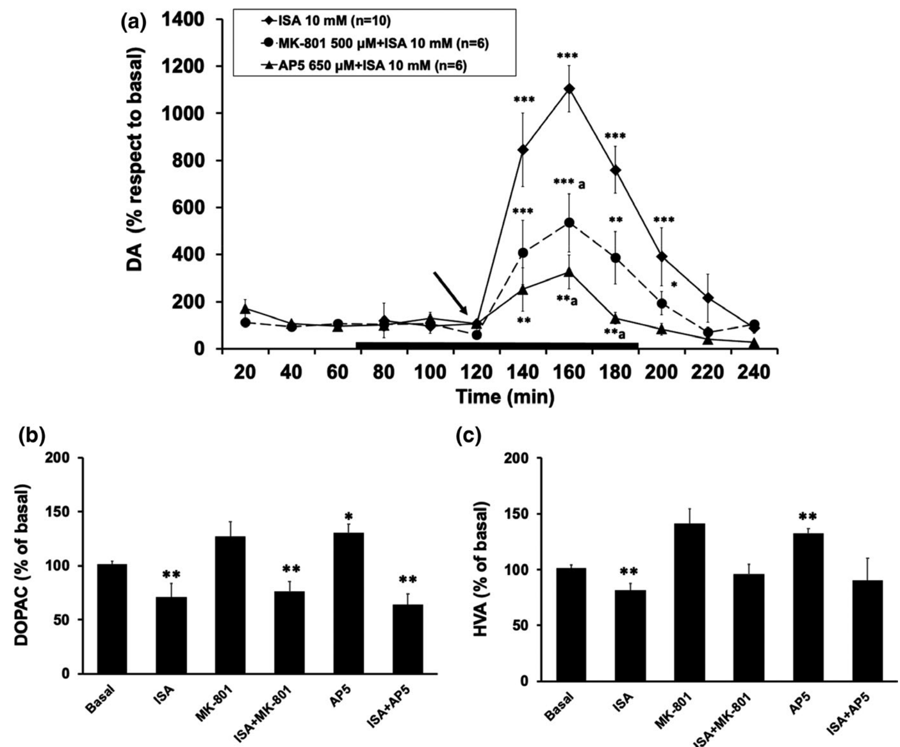

FIGURE 1 Effect of intrastriatal administration of isatin (10 mM) on the extracellular levels of dopamine (A), DOPAC (B) and HVA (C) in animals pretreated with MK-801 (500 μM) or AP5 (650 μM). The isatin was perfused for 60 min from the time indicated by the arrow. The perfusion of MK-801 or AP5 was performed for 120 min, shown by the black bar. The baseline was considered as the average of dopamine, DOPAC and HVA concentrations in the two samples collected before treatment administration. Significance levels: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, respect to basal levels (multiple range t test), and [a]$p < 0.05$, respect to 10 mM isatin control group (two-way repeated measures ANOVA followed by the Bonferroni post hoc test)

$F(11,220) = 27.72$, $p < 0.001$; interaction of Group × Time, $F(11,220) = 6.04$, $p < 0.001$]. Post hoc comparisons indicate significant differences between the effects of isatin alone and isatin + L-NAME or isatin + 7-NI ($p = 0.001$ and $p = 0.044$, respectively).

Figure 2B–C shows that administration of L-NAME does not significantly change the levels of the metabolites, and 7-NI produced significant increases in DOPAC and HVA levels up to a maximum of 139 ± 9.0% ($p = 0.0115$, t test) and 146.2% ± 16.4% ($p = 0.0299$, t test), relative to basal levels, respectively. The infusion of isatin to animals pretreated with L-NAME significantly decreased the DOPAC and HVA to 52% ± 16.7% ($p = 0.0063$, t test) and 58.8% ± 9.3% ($p = 0.00005$, t test), respectively, 80 min after the start of administration its infusion. The infusion of isatin + 7-NI significantly decreased the DOPAC to 50.4% ± 8.5% ($p = 0.0038$, t test) and does not significantly change the HVA levels. Besides, these declines did not differ significantly from the effect of isatin on both metabolites at the same time. The one-way ANOVA confirmed the existence of significant differences for the effects of L-NAME or 7-NI on DOPAC ($F(3,35) = 10.27$, $p < 0.001$, and $F(3,26) = 29.87$, $p < 0.001$, respectively) and HVA ($F(3,35) = 15.81$, $p < 0.001$, and $F(3,26) = 18.86$, $p < 0.001$, respectively). Post hoc comparisons indicate that the effects of administering isatin to animals pretreated with L-NAME or 7-NI do not differ significantly from those observed with the administration of isatin alone for DOPAC ($p = 1.00$ and $p = 1.00$, respectively) and HVA ($p = 0.258$ and $p = 1.00$, respectively).

3.4 | Effects of isatin on amino acid levels

The intrastriatal infusion of isatin also increased the striatal levels of the amino acids. In our experimental conditions, the basal levels of glutamate, aspartate, taurine and glycine were stable in the control group. Table 2 shows the mean of the concentrations of these substances in the two previously collected samples to the isatin infusion, which were considered as the basal levels. Table 2 also shows that the in situ perfusion of isatin significantly changed the extracellular levels of all substances, except for glycine.

Figure 3 shows the time-course of the isatin effect on the amino acid levels in the striatum. Intrastriatal infusion of isatin (10 mM) for one hour produced the following maximal increases in extracellular amino acids levels: glutamate up to 428.7% ± 127% ($p = 0.0023$, t test), aspartate up to 194.3% ± 29.6% ($p = 0.0011$, t test), and taurine up of 221 ± 22% ($p = 0.0065$, t test), always compared to basal levels. These increases occurred 40 min after starting administration of the isatin. Repeated measures ANOVA was made for the following groups: Ringer and isatin for glutamate [main effect of Group, $F(1,7) = 15.64$, $p = 0.005$; main effect of Time, $F(8,56) = 14.26$, $p < 0.001$; interaction of Group × Time, $F(8,56) = 12.26$, $p < 0.001$], Ringer and isatin for aspartate [main effect of Group, $F(1,7) = 0.98$, $p = 0.353$; main effect of Time, $F(8,56) = 3.63$, $p = 0.02$; interaction of Group × Time, $F(8,56) = 6.04$, $p < 0.073$], Ringer and isatin for taurine [main effect of Group, $F(1,7) = 2.30$, $p < 0.001$; main effect of Time,

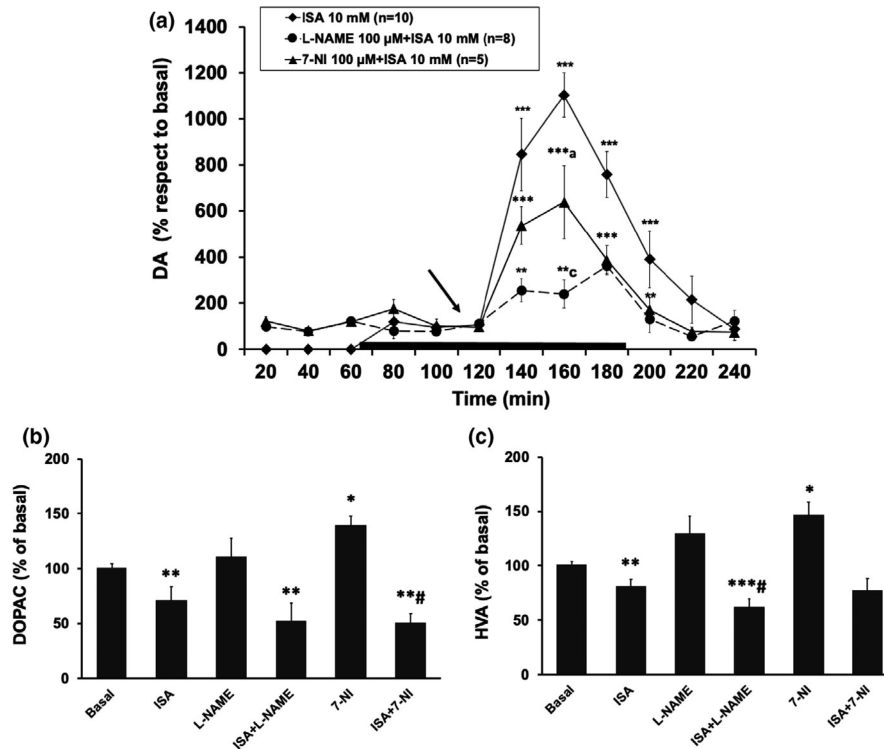

FIGURE 2 Effect of intrastriatal administration of isatin (10 mM) on the extracellular levels of dopamine (A), DOPAC (B) and HVA (C) in animals pretreated with L-NAME (100 μM) or 7-NI (100 μM). The isatin was perfused for 60 min from the time indicated by the arrow. The perfusion of MK-801 was performed for 120 min, shown by the black bar. The baseline was considered as the average of dopamine, DOPAC and HVA concentrations in the two samples collected before treatment administration. Significance levels: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, respect to basal levels (multiple range *t* test); $^{a}p < 0.05$, $^{c}p < 0.001$, respect to 10 mM isatin control group; and $^{\#}p < 0.05$, respect to L-NAME or 7-NI group (two-way repeated measures ANOVA followed by the Bonferroni post hoc test)

$F(8,56) = 3.97$, $p < 0.001$; interaction of Group × Time, $F(8,56) = 2.75$, $p < 0.012$], and Ringer and isatin for glycine [main effect of Group, $F(1,7) = 0.84$, $p < 0.389$; main effect of Time, $F(8,56) = 2.69$, $p < 0.014$; interaction of Group × Time, $F(8,56) = 1.74$, $p < 0.109$].

4 | DISCUSSION

The results obtained in the present study confirm our previous data by showing that isatin significantly increases the in vivo striatal dopamine release. Furthermore, we have demonstrated for the first time that the intrastriatal administration of isatin significantly increases the levels of glutamate and taurine in the rat striatum. The results also show a slight increase in the aspartate levels (194% ± 29.6%), but statistical analysis has not confirmed a clear effect of isatin on the extracellular levels of this amino acid in our experimental conditions (Figure 3B). However, the main finding of our study is that the isatin-induced dopamine release is partially dependent on the activation of the NMDARs and NO production in rat striatum. Taken together, these results indicate that isatin, in addition to acting on the dopaminergic system, can also alter other neurotransmitter systems related to the nigrostriatal pathway.

In addition to inhibiting MAO, isatin also acts as a releaser, stimulating the Ca^{++}-dependent dopamine release (Justo et al., 2016) and increasing the levels of other neurotransmitters such as acetylcholine, norepinephrine or serotonin (Glover et al., 1998; Minami et al., 1999, 2006). So, we might expect isatin to also be able to act on glutamatergic terminals inducing the increases in glutamate levels, although the mechanism through which isatin increases the glutamate and aspartate levels also remains to be determined.

By inhibiting MAO, isatin decreases the dopamine metabolism triggering increases in its extracellular levels (Justo et al., 2016; Minami et al., 1999, 2006). Thus, a hypothesis that would explain our results is that isatin-induced increases in the dopamine release stimulate the dopaminergic receptors, which could directly or indirectly modulate glutamate, aspartate, dopamine, acetylcholine and other neurotransmitters release in the striatum. It is important to consider that an increase in the glutamate release induced by isatin could also stimulate glutamatergic receptors located on dopaminergic terminals to induce increases in the dopamine release. However, this hypothesis was not evaluated in the present study and additional data are needed to determine the possible mechanisms by which isatin induces increases in extracellular levels of amino acids.

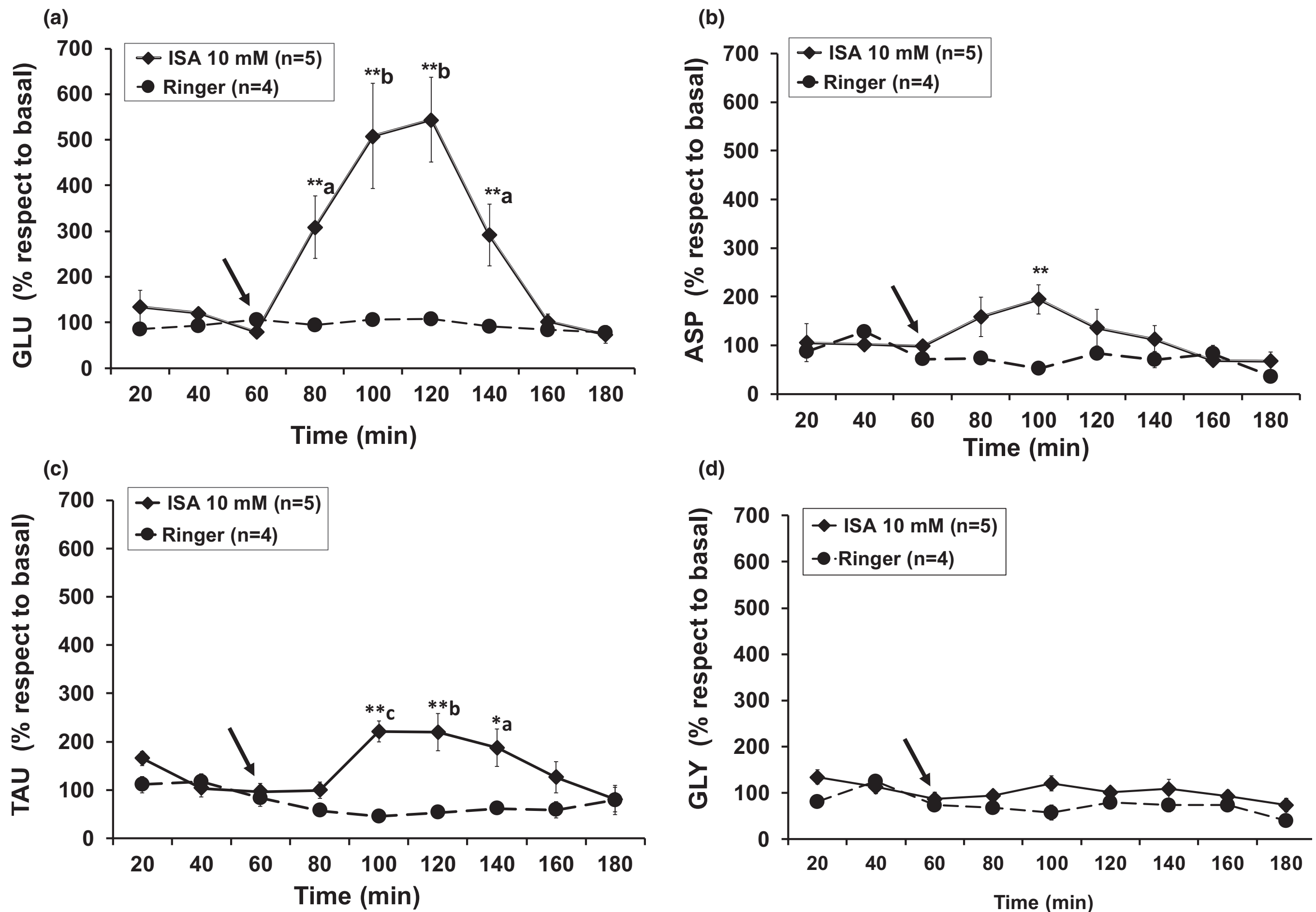

FIGURE 3 Effect of intrastriatal administration of (10 mM) isatin (ISA) on the extracellular levels of glutamate (GLU) (A), aspartate (ASP) (B), taurine (TAU) (C) and glycine (GLY) (D). The isatin was perfused for 60 min, through microdialysis probe, from the moment indicated by the arrow. The baseline was considered as the average of amino acids concentrations in the two samples collected before treatment administration. Significance levels: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$, respect to basal levels (multiple range *t* test), and $^{a}p < 0.05$, $^{b}p < 0.01$, and $^{c}p < 0.001$ respect to Ringer group (two-way repeated measures ANOVA followed by the Bonferroni post hoc test)

Taurine has been proposed as an inhibitory transmitter and neuroprotector in the nigrostriatal pathway, whose release increases responding to stimulation of NMDA receptors, depolarization with high K^+, or increases in glutamate levels (Bianchi et al., 1998; García Dopico et al., 2004). In this way, an isatin-induced increase in glutamate levels could, in turn, increase striatal taurine levels. This could be a possible hypothesis that would explain our results. Other possibilities include direct stimulation of taurine release induced by isatin or the increase in dopamine regulating the taurine levels, but these hypotheses were not evaluated in the present study. Regarding glycine, the data from the literature indicate the existence of very few glycinergic fibers in the nigrostriatal pathway and that the levels of glycine in the extracellular environment are mainly of a glial origin (Dopico et al., 2006). It should also be considered that the concentrations of amino acids observed in the striatum by microdialysis are questionable due to the high compartmentalization of these substances. Furthermore, Westerink et al. (1987) consider that the content of some amino acids in striatal dialysates could derive from metabolic processes and not from neuronal activity.

Considering the reciprocal modulation between dopaminergic and glutamatergic systems in the rat striatum, in other set of experiments we test the role of NMDARs activation on isatin-induced dopamine release. In our experimental conditions, pretreatment with MK-801 significantly decreased the isatin-induced dopamine release over 52%. The results obtained with AP5, a competitive antagonist, confirm the data observed with MK-801, because the increase in extracellular dopamine levels was significantly reduced in AP5 pretreated rats (70.5%) when compared with non-pretreated animals. These results indicate that the increases in the extracellular dopamine induced by isatin depend on activation of NMDARs in the rat striatum, at least in part.

Early studies on the effects of NMDARs agonists or antagonists on striatal dopamine release show contradictory results. However, many microdialysis studies show that the

administration of NMDA antagonists does not alter the basal levels of dopamine (David et al., 2005). Nevertheless, when the levels of dopamine were found increased, due to an inhibition of its reuptake or a stimulation of its release, the administration of NMDA antagonists significantly decreases the stimulated dopamine release (Weihmuller et al., 1992). In line with these data, our results show that infusion of MK-801 or AP5 did not change the basal dopamine levels, but the increases in dopamine levels induced by isatin were significantly decreased by a previous perfusion with NMDA antagonists. These results can suggest that, depending on the level of dopaminergic and glutamatergic activity, the activity of NMDARs may exert a facilitatory control on isatin-induced dopamine release in the striatum.

The exocytotic release of dopamine in the striatum depends on the influx of Ca^{++} through L and N type voltage-dependent calcium channels (VDCC), and also through NMDARs located on dopaminergic terminals (Cachope & Cheer, 2014). On the other hand, the release of dopamine evoked by isatin depends on the influx of Ca^{++}, and the removal of extracellular Ca^{++} significantly reduces the dopamine extracellular levels (Justo et al., 2016). Thus, a possible interpretation for our results would be that isatin, by inducing glutamate increases and NMDA activation, contributes to increase the dopamine overflow, and the pretreatment with NMDA antagonists reduces Ca^{++} influx through NMDARs and decreases the isatin-induced dopamine release observed by us.

So far, we have only discussed the possible role of presynaptic NMDARs activation in the dopamine release induced by isatin. However, as isatin increases both dopamine and glutamate levels in our experimental conditions, another hypothesis that we can postulate is that dopamine and glutamate could act on their postsynaptic receptors located on striatal interneurons to indirectly modulate striatal dopamine release.

Thus, in addition to studying the role of NMDARs in isatin-induced dopamine release, we also evaluated the role of NO production in this effect by pretreating animals with NOS inhibitors. Our results showed that pretreatment with L-NAME reduced isatin-induced dopamine overflow over 77%, while the 7-NI, a more specific neuronal NOS inhibitor, reduced release over 42%. These findings show that the increases in extracellular dopamine levels were also dependent on striatal NO production as it was significantly decreased by specific inhibitors.

Although the main target of corticostriatal and nigrostriatal terminals are the spiny projection neurons, a part of these terminals also synapses on striatal NOS-positive interneurons, which express NMDA, in addition to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate and metabotropic glutamate receptors (Salin et al., 1990; Vuillet et al., 1989; West et al., 2002), and also D1 dopamine receptors (Arcangeli et al., 2013; Lorenc-Koci & Czarnecka, 2013; Sammut et al., 2006). In these neurons, the stimulation of NMDARs increases Ca^{++} influx through VDCC, which binds to calmodulin. The activation of this Ca^{++}/calmodulin complex activates NOS and leads to an increase in NO levels (Brenmann & Bredt, 1997; Dawson & Dawson, 1996; Garthwaite & Boulton, 1995; Lorenc-Koci & Czarnecka, 2013; Schuman & Madison, 1994).

On the other hand, in addition to the NMDARs activation, the production of NO also depends on the activation of striatal D1-type dopamine receptors located on NOS-positive neurons (Hoque et al., 2010). In vivo studies have shown that stimulation of dopamine release or administration of D1 agonists significantly increases the NO production and efflux in the striatum, being these effects decreased by D1 antagonist and NOS inhibitors (Lorenc-Koci & Czarnecka, 2013). These data demonstrate the close interaction between D1 and NMDARs in nitrergic neurons, pointing out that NO production requires activation of both types of receptors.

Given that isatin significantly increases dopamine and glutamate overflow in the striatum and considering the convergence between dopaminergic and glutamatergic terminals on nitrergic neurons through D1 and NMDA receptors, the results obtained in the present study could indicate that the activation of these receptors increases the Ca^{++} influx into nitrergic neurons, NOS activation and NO production. However, our microdialysis results cannot demonstrate a direct relationship between increased dopamine or glutamate levels and increased NO production in the striatum. For this, we would have to administer a glutamatergic or dopaminergic antagonist together with the NOS inhibitor, plus isatin, to demonstrate the causal link between NO production and the activation of glutamatergic or dopamine receptors.

Once produced, NO diffuses into the extracellular medium and penetrates neighboring neurons where it produces its effects. Striatal dopaminergic terminals are an important target for this neurotransmitter, and previous in vivo studies show that NO facilitates the increases of extracellular dopamine levels by means of a Ca^{++}-dependent mechanism (Lorenc-Koci & Czarnecka, 2013; Rocchitta et al., 2004; Segovia & Mora, 1998; West et al., 2002). Other authors suggested that NO causes a reduction in the activity of dopamine transporter (DAT), thereby increasing extracellular dopamine levels (Kiss et al., 2004; Lonart & Johnson, 1994; Pogun et al., 1994).

Concerning dopamine metabolites, the results confirm our previous data by showing that isatin significantly decreased the extracellular levels of DOPAC and HVA, for basal levels (Justo et al., 2016). As previously commented, isatin is considered a reversible MAO inhibitor. The binding of isatin to human MAO-B is described as competitive, reversible and non-covalent. The Ki to this form is approximately 3 μM, while the Ki for MAO-A is 15 μM (Hubálek et al., 2005) and although isatin is generally described as a

selective MAO-B inhibitor, at higher doses, it can act on both MAO isoforms (Glover et al., 1998; Panova et al., 1997). This is because it has been observed that systemic administration of 200 mg/kg isatin significantly increased levels of norepinephrine and serotonin, which are preferential substrates for MAO-A (Medvedev et al., 1996). Therefore, at high doses, isatin could inhibit both forms of the enzyme and thus induce a much greater increase in dopamine release, with a total decrease in DOPAC and HVA levels. In our experimental conditions, 10 mM isatin, although significantly decreased the levels of the metabolites, did not produce a total decrease in these levels. We believe that this can be due to the low isatin concentration that crosses the membrane of the microdialysis probe (1.01%) and reaches the extracellular medium. Perhaps with higher concentrations of isatin, we could observe a total reduction in the levels of the metabolites, but this possibility was not evaluated in the present study.

The administration of isatin to animals pretreated with NMDARs antagonists or L-NAME did not change the levels of dopamine metabolites when compared to the effect of isatin alone. The exception was the 7-NI, which significantly decreased the effect of isatin on DOPAC and HVA levels compared to the isatin group. This was an unexpected result since previous studies in our laboratory show that the administration of 100 μM 7-NI did not modify the levels of the metabolites (Campos et al., 2006; Faro et al., 2012, 2013). Considering that the primary metabolites source comes from the metabolism, through MAO, of the cytoplasmic dopamine pool (Gazzara & Andersen, 1994; Zetterström et al., 1986), we interpret these results as follows: considering isatin as an MAO inhibitor, its administration leads to a decrease in the metabolism of dopamine with a consequent increase in its levels and a decrease in the levels of its metabolites. Furthermore, we have shown that isatin also acts as a releaser by stimulating exocytotic dopamine release by a mechanism dependent on terminal depolarization. In this way, blocking the NMDA receptors or inhibiting the NOS should not modify metabolites production in our experimental conditions, since newly synthesized dopamine, and not the dopamine released and again reuptake, is the primary source of DOPAC (Gazzara & Andersen, 1994; Zetterström et al., 1986), being the HVA levels mainly derived from DOPAC production.

5 | CONCLUSIONS

In the present study, we show that isatin, in addition to increasing the dopamine release, also increases glutamate and taurine levels in the striatum. Our results also show that treatment with NMDARs antagonist or NOS inhibition partially decreased, although not completely blocked, the effect of isatin on striatal dopamine release. This result could indirectly indicate that isatin could activate NMDA receptors and NO production, which may promote a further increase in the dopamine overflow. This could indicate that such mechanisms would be complementary and not the only ones that generate the effect of isatin on dopamine release.

ACKNOWLEDGEMENT

The research was supported by grant (Contract-Program) from University of Vigo (0022 122F641.02) and by a scholarship.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Lilian R. F. Faro designed the study, analyzed the data, wrote and reviewed the manuscript; Lorenzo Justo collected, analyzed the data and reviewed the manuscript; Raquel Gómez collected and analyzed the data. Rafael Durán designed the study and reviewed the manuscript.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ejn.15319.

DATA AVAILABILITY STATEMENT

Data from the effects of intrastriatal administration of isatin together with MK-801 on the dopamine levels in animals pretreated with isatin; the effect of administration of isatin together with 7-NI on the dopamine levels in animals pretreated with isatin; and the effect of isatin + KCl on the dopamine levels in animals pretreated with isatin will be made available upon request.

ORCID

Lilian R. F. Faro https://orcid.org/0000-0002-6768-0691

REFERENCES

Alfonso, M., Durán, R., Campos, F., Perez-Vences, D., Faro, L. R., & Arias, B. (2003). Mechanisms underlying domoic acid-induced dopamine release from striatum: An in vivo microdialysis study. *Neurochemical Research*, *28*, 1487–1493. https://doi.org/10.1023/a:1025614223684

Arcangeli, S., Tozzi, A., Tantucci, M., Spaccatini, C., de Iure, A., Costa, C., Di Filippo, M., Picconi, B., Giampà, C., Fusco, F. R., Amoroso, S., & Calabresi, P. (2013). Ischemic-LTP in striatal spiny neurons of both direct and indirect pathway requires the activation of D1-like receptors and NO/soluble guanylate cyclase/cGMP transmission. *Journal of Cerebral Blood Flow and Metabolism*, *33*, 278–286. https://doi.org/10.1038/jcbfm.2012.167

Arias, B., Durán, R., & Alfonso, M. (1998). In vivo release of dopamine and its metabolites from rat striatum in response to domoic acid. *Neurochemical Research*, *23*, 1509–1514. https://doi.org/10.1023/a:1020919818652

Bianchi, L., Colivicchi, M. A., Bolam, J. P., & Della Corte, L. (1998). The release of amino acids from rat neostriatum and substantia nigra in vivo: A dual microdialysis probe analysis. *Neuroscience*, *87*, 171–180. https://doi.org/10.1016/s0306-4522(98)00090-6

Brenmann, J. E., & Bredt, D. S. (1997). Synaptic signalling by nitric oxide. *Current Opinion in Neurobiology*, *7*, 374–378. https://doi.org/10.1016/s0959-4388(97)80065-7

Cachope, R., & Cheer, J. F. (2014). Local control of striatal dopamine release. *Frontiers in Behavioural Neurosciences*, *8*, 1–7. https://doi.org/10.3389/fnbeh.2014.00188

Campos, F., Alfonso, M., Vidal, L., Faro, L. R., & Durán, R. (2006). Mediation of glutamatergic receptors and nitric oxide on striatal dopamine release evoked by anatoxin-a. An in vivo microdialysis study. *European Journal of Pharmacology*, *548*, 90–98. https://doi.org/10.1016/j.ejphar.2006.07.044

David, H. N., Ansseau, M., & Abraini, J. H. (2005). Dopamine–glutamate reciprocal modulation of release and motor responses in the rat caudate–putamen and nucleus accumbens of ''intact'' animals. *Brain Research Reviews*, *50*, 336–360. https://doi.org/10.1016/j.brainresrev.2005.09.002

Dawson, V. L., & Dawson, T. M. (1996). Nitric oxide actions in neurochemistry. *Neurochemistry International*, *29*, 97–110. https://doi.org/10.1016/0197-0186(95)00149-2

Dopico, J. G., González-Hernández, T., Pérez, I. M., García, I. G., Abril, A. M., Inchausti, J. O., & Rodríguez, D. M. (2006). Glycine release in the substantia nigra: Interaction with glutamate and GABA. *Neuropharmacology*, *50*(5), 548–557. https://doi.org/10.1016/j.neuropharm.2005.10.014

Egenrieder, L., Mitricheva, E., Spanagel, R., & Noori, H. R. (2020). No basal or drug-induced sex differences in striatal dopaminergic levels: A cluster and meta-analysis of rat microdialysis studies. *Journal of Neurochemistry*, *152*, 482–492. https://doi.org/10.1111/jnc.14911

Faro, L. R., Alfonso, M., Maués, L. A., & Durán, R. (2012). Role of ionotropic glutamatergic receptors and nitric oxide in the effects of flutriafol, a triazole fungicide, on the in vivo striatal dopamine release. *Journal of Toxicological Sciences*, *37*, 1135–1142. https://doi.org/10.2131/jts.37.1135

Faro, L., do Nascimento, J., Alfonso, M., & Durán, R. (2002). Protection of methylmercury effects on the in vivo dopamine release by NMDA receptor antagonists and nitric oxide synthase inhibitors. *Neuropharmacology*, *42*, 612–618. https://doi.org/10.1016/s0028-3908(02)00009-6

Faro, L. R. F., Justo, L. A., Alfonso, M., & Durán, R. (2020). Possible synergies between isatin, an endogenous MAO inhibitor, and antiparkinsonian agents on the dopamine release from striatum of freely moving rats. *Neuropharmacology*, *171*, 108083. https://doi.org/10.1016/j.neuropharm.2020.108083

Faro, L. R. F., Nunes, B. V., Alfonso, M., Ferreira, V. M., & Duran, R. (2013). Role of glutamate receptors and nitric oxide on the effects of glufosinate ammonium, an organophosphate pesticide, on in vivo dopamine release in rat striatum. *Toxicology*, *311*, 154–161. https://doi.org/10.1016/j.tox.2013.06.008

García Dopico, J., Perdomo Díaz, J., Alonso, T. J., González Hernández, T., Castro Fuentes, R., & Rodríguez Díaz, M. (2004). Extracellular taurine in the substantia nigra: Taurine-glutamate interaction. *Journal of Neuroscience Research*, *76*, 528–538. https://doi.org/10.1002/jnr.20108

Garthwaite, J., & Boulton, C. L. (1995). Nitric oxide signaling in the central nervous system. *Annual Review of Physiology*, *57*, 683–706. https://doi.org/10.1146/annurev.ph.57.030195.003343

Gazzara, R. A., & Andersen, S. L. (1994). Calcium dependency and tetrodotoxin sensitivity of neostriatal dopamine release in 5-day-old and adult rats as measured by in vivo microdialysis. *Journal of Neurochemistry*, *62*, 1741–1749. https://doi.org/10.1046/j.1471-4159.1994.62051741.x

Glover, V., Bhattacharya, S., Chakrabarti, A., & Sandler, M. (1998). The psychopharmacology of isatin: A brief review. *Stress Med*, *14*, 225–229. https://doi.org/10.1002/(SICI)1099-1700(199810)14:4<225:AID-SMI801>3.0.CO;2-P

Hamaue, N., Minami, M., Hirafuji, M., Terado, M., Machida, M., Yamazaki, N., Yoshioca, M., Ogata, A., & Tashito, K. (1999). Isatin, an endogenous MAO inhibitor, as a new biological modulator. *CNS Drug Reviews*, *5*, 331–346.

Hamaue, N., Minami, M., Terado, M., Hirafuji, M., Endo, T., Machida, M., Hiroshige, T., Ogata, A., Tashiro, K., Saito, H., & Parvez, S. H. (2004). Comparative study of the effects of isatin, an endogenous MAO- inhibitor, and selegiline on bradykinesia and dopamine levels in a rat model of Parkinson's disease induced by the Japanese encephalitis virus. *Neurotoxicology*, *25*, 205–213. https://doi.org/10.1016/S0161-813X(03)00100-1

Hoque, K. E., Indorkar, R. P., Sammut, S., & West, A. R. (2010). Impact of dopamine–glutamate interactions on striatal neuronal nitric oxide synthase activity. *Psychopharmacology (Berl)*, *207*, 571–581. https://doi.org/10.1007/s00213-009-1687-0

Hubálek, F., Binda, C., Khalil, A., Li, M., Mattevi, A., Castagnoli, N., & Edmondson, D. E. (2005). Demonstration of isoleucine 199 as a structural determinant for the selective inhibition of human monoamine oxidase B by specific reversible inhibitors. *Journal of Biological Chemistry*, *280*, 15761–21576. https://doi.org/10.1074/jbc.M500949200

Justo, L. A., Duran, R., Alfonso, M., Fajardo, D., & Faro, L. R. F. (2016). Effects and mechanism of action of isatin, and MAO inhibitor, on in vivo striatal dopamine release. *Neurochemistry International*, *99*, 147–157. https://doi.org/10.1016/j.neuint.2016.06.012

Kiss, J. P., Zsilla, G., & Vizi, E. S. (2004). Inhibitory effect of nitric oxide on dopamine transporters: Interneuronal communication without receptors. *Neurochemistry International*, *45*, 485–489. https://doi.org/10.1016/j.neuint.2003.11.004

Lonart, G., & Johnson, K. M. (1994). Inhibitory effects of nitric oxide on the uptake of [^{3}H]dopamine and [^{3}H]glutamate by striatal synaptosomes. *Journal of Neurochemistry*, *63*, 2108–2117. https://doi.org/10.1046/j.1471-4159.1994.63062108.x

Lorenc-Koci, E., & Czarnecka, A. (2013). Role of nitric oxide in the regulation of motor function. An overview of behavioral, biochemical and histological studies in animal models. *Pharmacological Reports*, *65*, 1043–1055. https://doi.org/10.1016/s1734-1140(13)71464-6

Manley-King, C. I., Bergh, J. J., & Petzer, J. P. (2011). Inhibition of monoamine oxidase by selected C5-and C6-substituted isatin analogues. *Bioorganic & Medicinal Chemistry*, *19*, 261–274. https://doi.org/10.1016/j.bmc.2010.11.028

Medvedev, A. E., Buneeva, O., Gnedenko, O., Ershov, P., & Ivanov, A. (2018). Isatin, an endogenous nonpeptide biofactor: A review of its molecular targets, mechanisms of actions, and their biomedical implications. *BioFactors*, *44*, 95–108. https://doi.org/10.1002/biof.1408

Medvedev, A. E., Clow, A., Sandler, M., & Glover, V. (1996). Isatin: A link between natriuretic peptides and monoamines? *Biochemical Pharmacology*, *52*, 385–391. https://doi.org/10.1016/0006-2952(96)00206-7

Medvedev, A. E., & Glover, V. (2004). Tribulin and endogenous MAO-inhibitory regulation in vivo. *Neurotoxicology*, *25*, 185–192. https://doi.org/10.1016/S0161-813X(03)00098-6

Medvedev, A. E., Igosheva, N., Crumeyrolle-Arias, M., & Glover, V. (2005). Isatin: Role in stress and anxiety. *Stress*, *8*, 175–183. https://doi.org/10.1080/10253890500342321

Minami, M., Hamaue, N., Endo, T., Hirafuji, M., Terado, M., Ide, H., Yamazaki, N., Yoshioka, M., Ogata, A., & Tashiro, K. (1999). Effects of isatin, an endogenous MAO inhibitor, on dopamine (DA) and acetylcholine (ACh) concentrations in rats. *Nihon Yakurigaku Zasshi*, *114*, 186P–191P. https://doi.org/10.1254/fpj.114.supplement_186

Minami, M., Hamaue, N., Hirafuji, M., Saito, H., Hiroshige, T., Ogata, A., Tashiro, K., & Parvez, S. H. (2006). Isatin, an endogenous MAO inhibitor, and a rat model of Parkinson's disease induced by the Japanese encephalitis virus. *Journal of Neural Transmission. Supplementum*, *71*, 87–95. https://doi.org/10.1007/978-3-211-33328-0_10

Ogata, A., Hamaue, N., Terado, M., Minami, M., Nagashima, K., & Tashiro, K. (2003). Isatin, an endogenous MAO inhibitor, improves bradykinesia and dopamine levels in a rat model of Parkinson's disease induced by Japanese encephalitis virus. *Journal of the Neurological Sciences*, *206*, 79–83. https://doi.org/10.1016/s0022-510x(02)00342-8

Panova, N.G., Zemskova, M.A., Axenova, L.N., & Medvedev, A.E. (1997). Does isatin interact with rat brain monoamine oxidases in vivo?. *Neuroscience Letters*, *233*, 58–60. https://doi.org/10.1016/s0304-3940(97)00597-1.

Pogun, S., Baumann, M. H., & Kuhar, M. J. (1994). Nitric oxide inhibits [$3H^+$] dopamine uptake. *Brain Research*, *641*, 83–89. https://doi.org/10.1016/0006-8993(94)91818-x

Rocchitta, G., Migheli, R., Mura, M. P., Esposito, G., Desole, M. S., Miele, E., Miele, M., & Serra, P. A. (2004). Signalling pathways in the nitric oxide donor-induced dopamine release in the striatum of freely moving rats: Evidence that exogenous nitric oxide promotes Ca^{2+} entry through store-operated channels. *Brain Research*, *1023*, 243–252. https://doi.org/10.1016/j.brainres.2004.07.040

Salin, P., Kerkerian-Le Goff, L., Heidet, V., Epelbaum, J., & Nieoullon, A. (1990). Somatostatin-immunoreactive neurons in the rat striatum: Effects of corticostriatal and nigrostriatal dopaminergic lesions. *Brain Research*, *521*, 23–32. https://doi.org/10.1016/0006-8993(90)91520-q

Sammut, S., Dec, A., Mitchell, D., Linardakis, J., Ortiguela, M., & West, A. R. (2006). Phasic dopaminergic transmission increases NO efflux in the rat dorsal striatum via a neuronal NOS and a dopamine D(1/5) receptor-dependent mechanism. *Neuropsychopharmacol*, *31*, 493–505. https://doi.org/10.1038/sj.npp.1300826

Schuman, E. M., & Madison, D. V. (1994). Nitric oxide and synaptic function. *Annual Review of Neuroscience*, *17*, 153–183. https://doi.org/10.1146/annurev.ne.17.030194.001101

Segovia, G., & Mora, F. (1998). Role of nitric oxide in modulating the release of dopamine, glutamate, and GABA in striatum of the freely moving rat. *Brain Research Bulletin*, *45*, 275–279. https://doi.org/10.1016/s0361-9230(97)00402-4

Vuillet, J., Kerkerian, L., Kachidian, P., Bosler, O., & Nieoullon, A. (1989). Ultrastructural correlates of functional relationships between nigral dopaminergic or cortical afferent fibers and neuropeptide Y-containing neurons in the rat striatum. *Neuroscience Letters*, *100*, 99–104. https://doi.org/10.1016/0304-3940(89)90667-8

Weihmuller, F. B., O'Dell, S. J., & Marshall, J. F. (1992). MK-801 protection against methamphetamine-induced striatal dopamine terminal injury is associated with attenuated dopamine overflow. *Synapse (New York, N. Y.)*, *11*, 155–163. https://doi.org/10.1002/syn.890110209

West, A. R., Galloway, M. P., & Grace, A. A. (2002). Regulation of striatal dopamine neurotransmission by nitric oxide: Effector pathways and signaling mechanisms. *Synapse (New York, N. Y.)*, *44*, 227–245. https://doi.org/10.1002/syn.10076

Westerink, B., Damsma, G., Rollema, H., De Vries, J., & Horn, A. (1987). Scope and limitations of in vivo brain dialysis: A comparison of its application to various neurotransmitter systems. *Life Sciences*, *41*, 1763–1776. https://doi.org/10.1016/0024-3205(87)90695-3

Zetterström, T., Brundin, P., Gage, F. H., Sharp, T., Isacson, O., Dunnett, S. B., Ungerstedt, U., & Björklund, A. (1986). In vivo measurement of spontaneous release and metabolism of dopamine from intrastriatal nigral grafts using intracerebral dialysis. *Brain Research*, *362*, 344–349. https://doi.org/10.1016/0006-8993(86)90460-9

Zhou, Y., Zhao, Z., & Xie, J. (2001). Effects of isatin on rotational behavior and DA levels in caudate putamen in parkinsonian rats. *Brain Research*, *917*, 127–132. https://doi.org/10.1016/s0006-8993(01)02935-3

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Faro LRF, Justo L, Gómez R, Durán R. Participation of glutamatergic and nitrergic systems in the striatal dopamine release induced by isatin, a MAO inhibitor. *Eur J Neurosci*. 2021;00:1–11. https://doi.org/10.1111/ejn.15319