

# Nesfatin-1 exerts anticonvulsant effect by reducing oxidative stress in experimental epilepsy model

Seval Musuroglu Keloglan<sup>1\*</sup>, Fatma Banu Aycik<sup>2</sup>, Suleyman Emre Kocacan<sup>2</sup>, Burak Yazgan<sup>3</sup>,  
Mustafa Ayyildiz<sup>2</sup>, Erdal Agar<sup>2</sup>

<sup>1</sup> Department of Physiology, Faculty of Medicine, University of Adiyaman, Adiyaman, Turkey,

<sup>2</sup> Department of Physiology, Faculty of Medicine, University of Ondokuz Mayıs, Samsun, Turkey,

<sup>3</sup> Department of Medical Services and Techniques, Sabuncuoğlu Serefeddin Health Services Vocational School,  
University of Amasya, Amasya, Turkey,

\*Email: sevalkeloglan@hotmail.com

Neuropeptides play an important role in the pathogenesis of epilepsy. In the present study, the effect of nesfatin-1, a neuropeptide, was investigated on penicillin-induced epilepsy model. Epileptiform activity was induced by an injection of penicillin into the somatomotor cortex at 56 albino Wistar rats. Nesfatin-1 (i.c.v.) was administered at five different doses (12.5, 25, 50, 100, and 200 pmol) 30 min after a penicillin administration. Astressin 2B, a corticotropin-releasing factor (CRF) receptor antagonist, was administered 10 minutes later the effective dose of nesfatin-1 (50 pmol, i.c.v.). Superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and malondialdehyde (MDA) levels in cerebrum were analysed by ELISA method. Nesfatin-1, at the doses of 25, 50 and 100 pmol, significantly reduced the frequency of epileptiform activity. However, none of the doses of nesfatin-1 had any effect on the amplitude of epileptiform activity. Astressin 2B alone did not show any effect on epileptiform activity. In addition, astressin 2B had no effect on the anticonvulsant effect of nesfatin-1. Nesfatin-1 (at the doses of 25, 50, 100 pmol) did not alter SOD and GSH levels, but significantly increased the GPx and GR levels. Nesfatin-1 (at a dose of 50 pmol) significantly decreased the MDA level in the cerebrum. Nesfatin-1 shows anticonvulsant effect and astressin 2B did not affect the anticonvulsant effect of nesfatin-1. We suggest that nesfatin-1 has oxidative stress-mediated anticonvulsant effect in the penicillin-induced epileptic activity.

**Key words:** neuropeptide, nesfatin-1, epilepsy, penicillin, oxidative stress

## INTRODUCTION

Epilepsy is a disease characterized by an imbalance between inhibitory and excitatory neuronal inputs. Neuropeptides may contribute to this imbalance between inhibitory and excitatory neurotransmitters (Clynen et al., 2014). Nesfatin-1, is corresponding to NEFA/nucleobindin2 (NUCB2), a neuropeptide defined as a satiety molecule in the hypothalamus (Oh-l et al., 2006). The inhibitory effect of nesfatin-1 on feeding may be mediated by hyperpolarization of neuropeptide Y neurons in the arcuate nucleus (Price et al., 2008).

Nesfatin-1 affects neuronal excitability, and may cause hyperpolarization of most nigral dopaminergic neurons (Li et al., 2014). The effects of nesfatin-1 on neuronal excitability have led to its role in neurological disorders such as epilepsy (Dore et al., 2017).

Aydın et al. (2009) found that patients with primary generalized epilepsy had high saliva and serum nesfatin-1 levels, which decreased with antiepileptic treatment. Another study found that the plasma level of nesfatin-1 remained higher than the seizure activity for five min, then decreased, and after 48 h it decreased to 50% of the normal value (Aydın et al., 2011). A study conducted in mice demonstrated that there

was a significant increase in serum nesfatin-1 levels in acute pentylenetetrazole (PTZ) and PTZ kindling groups (Erkeç et al., 2018). It has been suggested that nesfatin-1 may be a sensitive marker of epilepsy due to these levels of nesfatin-1 seen in epileptic patients and rats (Aydın et al., 2009; Liu et al., 2011; Pałasz et al., 2012). However, in these studies, it is not clear whether the increase in the level of nesfatin-1 serum and saliva has a protective role against seizures or whether it is an agent that triggers the seizure. The answer to this question will perhaps be more easily elucidated in the future by the identification of the receptor of nesfatin-1. Although the receptor of nesfatin-1 is not precisely defined, it may have a relationship with the corticotropin-releasing factor (CRF) signaling system. In one study, the CRF2 antagonist astressin 2B abolished the anorexigenic effect of nesfatin-1 in rats (Stengel et al., 2009). Therefore, the current study also investigated CRF2 specific receptor antagonist astressin 2B, given this relationship of nesfatin-1 with CRF.

Oxidative stress and mitochondrial dysfunction play a role in the pathogenesis of most neurodegenerative diseases, including epilepsy (Lin and Beal, 2006; Rowley and Patel, 2013). Excessive free radical production due to neuronal hyperexcitability and oxidative damage may play an important role in the initiation and progression of epilepsy (Geronzi et al., 2018). Patients with epilepsy showed a high level of lipid peroxidation markers, while the activity of antioxidant defense system was low (Fedin et al., 2019). Mitochondrial antioxidant superoxide dismutase (SOD2) deficient mice show spontaneous motor seizures and neuronal death (Liang et al., 2012). The increase in the activities of enzymes such as glutathione peroxidase (GPx) and glutathione reductase (GR) in mitochondria has a neuroprotective effect against oxidative damage in patients with epilepsy (Ristic et al., 2015). GPx enzyme catalyzes hydroperoxide reduction in cells using glutathione (GSH) (Aguiar et al., 2012). An indicator of oxidative damage in epilepsy is elevated malondialdehyde (MDA) levels (Sun et al., 2022). MDA has found to be increased in the hippocampus in rats in the pilocarpine-induced epilepsy model (Wang et al., 2019). These data show that antioxidant enzymes are associated with epilepsy and that an increase or decreased in their activity may be neuroprotective in epilepsy.

There are studies demonstrating that the neuroprotective role of nesfatin-1 may be associated with anti-apoptosis, anti-inflammation, and antioxidant stress (Dong et al., 2019; Altas et al., 2022). Nesfatin-1 treatment was found to improve subarachnoid hemorrhage-induced neurological impairment and oxidative brain injury (Özsavcı et al., 2011). In the study of Shen et al. (2017), nesfatin-1 was shown to have a neuro-

protective effect by reducing the loss of Parkinson's disease nigral dopaminergic neurons. These findings suggest that nesfatin-1 may have oxidative stress-mediated neuroprotective effects in epilepsy, a neurodegenerative disease.

Even though there are studies that link nesfatin-1 with epilepsy, its role in epilepsy is unclear. This study primarily aimed to investigate for the first time whether nesfatin-1 is an anticonvulsant or a proconvulsant agent; secondly, the effect of the CRF2 receptor antagonist astressin 2B on the possible effect of nesfatin in epilepsy; and thirdly, the effect of oxidative stress parameters on the possible effects of nesfatin-1 in epilepsy.

## METHODS

### Animals and procedure

The study was carried out with 56 male Wistar Albino rats weighing 180–220 grams. All animals were maintained in a temperature-controlled environment with a 12-hour light-dark cycle with free access to tap water and food. The local ethics committee approved all experimental procedures. The experimental groups were constructed as follows: Penicillin control (500 IU, i.c.); Penicillin + Nesfatin-1 (12.5 pmol, i.c.v.); Penicillin + Nesfatin-1 (25 pmol, i.c.v.); Penicillin + Nesfatin-1 (50 pmol, i.c.v.); Penicillin + Nesfatin-1 (100 pmol, i.c.v.); Penicillin + Nesfatin-1 (200 pmol, i.c.v.); Penicillin + Astressin 2B (30 µg, i.c.v.); Penicillin + Nesfatin-1 (50 pmol, i.c.v.) + Astressin-2B (30 µg, i.c.v.). The each group was composed of seven rats.

### Placement of electrodes for electrocorticography recordings

The rats were anesthetized with urethane (1.25 g/kg, i.p.) and placed in the stereotaxic apparatus. Two screw electrodes were placed on the left somatomotor cortex by taking the bregma reference point and in accordance with the stereotaxic coordinates (Kozan et al., 2006). Penicillin-G was injected into the cerebral cortex (coordinates: AP -2.0 mm, LL +2.0 mm, DV -1.0 mm) and nesfatin-1, astressin 2B were injected lateral ventricle (coordinates: AP -1.0 mm, LL +1.5 mm, DV -3.2 mm), taking bregma as the reference point.

Then, a bipolar electrode was connected to a computerized ECoG recording system by an isolated flexible cable, ECoG activity was continuously monitored (PowerLab, 8/SP, AD Instruments, Castle Hill, NSW, Australia). The frequencies and amplitudes of the ECoG

activity were measured offline with the Labchart 7 Pro (AD Instruments, Australia). The spikes were counted every 10 min for 180 min via the software's spike shape detection. The amplitudes of the spikes were calculated with the software's average cyclic height feature. Epileptiform activity was started after approximately recording 2 min basal activity. The data was then transferred to an Excel programme to evaluate the mean spike frequency and amplitude of epileptiform activity via the following formula:

Frequency or amplitude value % =  $100 \times (\text{the mean spike frequency or amplitude after substance administered} / \text{the mean of spike frequency or amplitude before substance administered})$

### Drug administration

Sterile physiological normal saline, penicillin-G potassium (I.E. Ulugay, Turkey), nesfatin-1 and astressin 2B (Sigma Chemical and Tocris) were used. Penicillin G (500 IU) potassium was dissolved in normal saline, and 2 µl was injected into the 1 mm beneath the brain surface. Nesfatin-1 and astressin 2B (i.c.v.) were dissolved with sterile physiological saline and 1 µl of the required doses was administered. Nesfatin-1 and astressin 2B doses were determined in accordance with previous studies (Stengel et al., 2009; Tanida et al., 2015). The doses of nesfatin-1 (12.5 pmol, 25 pmol, 50 pmol, 100 pmol, 200 pmol) were administered 30 min after the i.c. application of penicillin. Astressin 2B (30 µg) dose were administered 30 min after the application of penicillin or 10 min after the application of nesfatin-1 (50 pmol) were administered. The drug solutions were injected at an infusion rate of 0.5 µl/min, using a Hamilton microsyringe (Aldrich, Milwaukee, WI, USA), and the needle remained in place for an additional minute to prevent backflow of the drug and their brain tissue was removed. The brains were stored at -80°C and used for biochemical analysis.

### Biochemical analysis

Superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and malondialdehyde (MDA) activities were quantitatively evaluated by ELISA method [optical density (OD) at 450 nm]. 100 mg of brain tissues were separately homogenized in appropriate buffers which are respectively contain SOD buffer (20 mM HEPES, 1 mM EGTA, 210 mM mannitol, 70 mM sucrose, pH: 7.2), GSH (MES buffer), GPx buffer (50 mM Tris HCL,

5 mM EDTA, 1 mM DTT, pH: 7.5), GR buffer (50 mM potassium phosphate, 1 mM EDTA, pH: 7.5) and MDA (RIPA buffer, cell signaling) by using an ultraturax homogenizator (IKA) at 20.000 rpm for 60 s and centrifuged at 15.000 rpm for 10 min. SOD (Cayman Chemical), GSH (Cayman Chemical), GPx (Cayman Chemical), GR (Cayman Chemical) and MDA (Cayman Chemical) levels in the supernatants obtained from brain tissues determined by colorimetric methods according to the manufacturer's instructions. All samples and standards were prepared duplicate and the colorimetric measurement by using spectrophotometer (Multiscan Go, Thermo). Lipid peroxidation and antioxidant enzyme activity levels of the samples were calculated against the standard curve.

### Statistical analysis

Statistical comparisons were made using SPSS 17.0. The normality of the data was tested using the Shapiro-Wilk test before analyses. After verifying that the data obtained from electrophysiological recordings were normally distributed, one-way analysis of variance and Tukey-Kramer *post hoc* tests for multiple comparisons were performed.  $p < 0.05$  was considered statistically significant.

## RESULTS

Epileptiform activity was induced by administering 500 IU penicillin-G intracortically. Spike activity began within 2-4 min after penicillin injection; It reached a stable level as to frequency and amplitude within 30 min and lasted 3 h. The means of the spike frequency and amplitude were  $41.47 \pm 2.59$  spike/min and  $956.62 \pm 89.53$  µV, respectively, in the control group after 120 min the penicillin injection (Fig. 1A).

Five doses of nesfatin-1 (12.5, 25, 50, 100 and 200 pmol, i.c.v.) were administered after the penicillin injection. Nesfatin-1, at doses of 12.5 and 200 pmol, did not affect the mean frequency and amplitude of penicillin induced epileptiform activity ( $p > 0.05$ ) (Fig. 2A, 2B). Nesfatin-1 at doses of 25, 50 and 100 pmol significantly decreased the mean frequency of epileptiform activity without changing the amplitude. 25 pmol nesfatin-1 significantly decreased the mean spike frequency in between 70-90 and 170-180 min ( $p < 0.05$ ). 50 pmol nesfatin-1 significantly decreased the mean spike frequency in between 10-180 min ( $p < 0.05$ ) (Fig. 2A). Nesfatin-1 (100 pmol) significantly decreased the mean spike frequency in between 50-180 min ( $p < 0.05$ ). Nesfatin-1, a dose of 50 pmol,

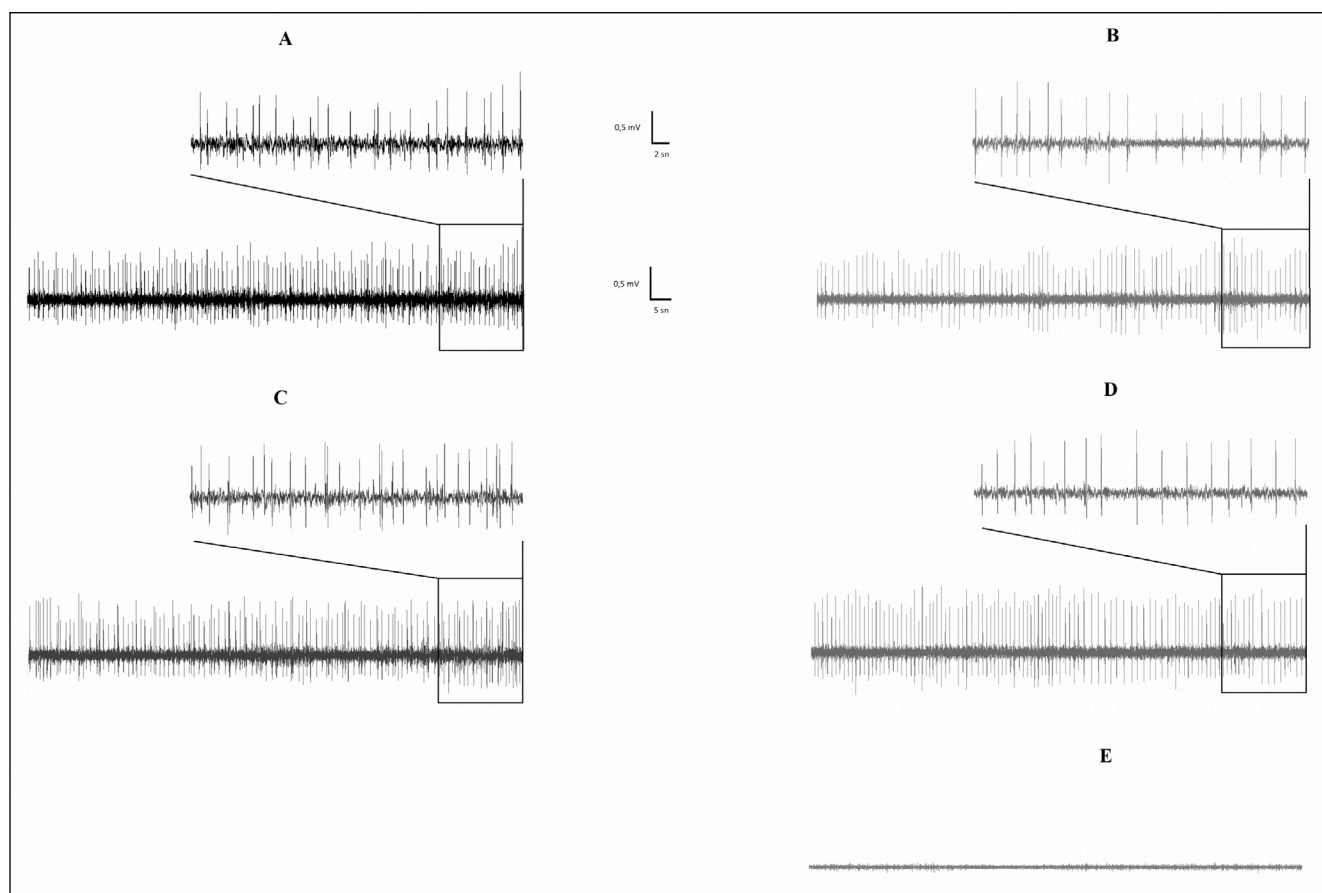


Fig. 1. (A) Penicillin (500 IU) induced epileptiform activity on ECoG. (B) The intracerebroventricular (i.c.v.) administration of nesfatin-1 (50 pmol), decreased the mean frequency of penicillin-induced epileptiform activity without changing the mean amplitude. (C) Administration of the astressin 2B (i.c.v.), at a dose of 30 µg, did not affect the mean frequency and amplitude of penicillin induced epileptiform activity. (D) Administration of the astressin 2B (30 µg, icv) 10 min after nesfatin-1 (50 pmol) showed that the anticonvulsant effect of nesfatin-1 (50 pmol) was maintained (E) Baseline ECoG activity before penicillin or the injection of other substances. Representative ECoGs are presented for the 120 min after penicillin administration.

was considered effective for to reduce epileptic activity and used for further experiments. The means of the spike frequency and amplitude were  $28.96 \pm 7.27$  spike/min and  $930.49 \pm 38.60$  µV, respectively, in the nesfatin-1 group (50 pmol) after 120 min the penicillin injection (Fig. 1B).

Astressin 2B (30 µg, i.c.v.) were administered after the penicillin injection. Astressin 2B did not affect the mean frequency and amplitude of penicillin induced epileptiform activity ( $p > 0.05$ ) (Fig. 3A and B). The means of the spike frequency and amplitude were  $45.49 \pm 2.45$  spike/min and  $1104.41 \pm 242.47$  µV, respectively, in the astressin 2B group after 120 min the penicillin injection (Fig. 1C).

Astressin 2B administration (30 µg) 10 min after the nesfatin-1 injection (50 pmol) significantly decreased the mean frequency of epileptiform activity without changing the amplitude in between 10–180 minutes compared to the penicillin-injected group ( $p < 0.05$ )

(Fig. 3A, 3B). The mean spike frequency and amplitude were  $32.32 \pm 3.44$  spike/min and  $1061.42 \pm 205.84$  µV, respectively, in the interaction group (nesfatin-1, 50 pmol + Astressin 2B, 30 µg) after 120 min the penicillin injection (Fig. 1D).

### Determination of biochemical analysis

Table 1 shows the biochemical analysis for the cerebrum of penicillin control and nesfatin-1 dose groups. Nesfatin-1 (25, 50, 100 pmol) injection did not alter SOD and GSH levels in the cerebrum ( $p > 0.05$ ). The GR and GPx levels in the cerebrum significantly were increased in the nesfatin-1 (25, 50, 100 pmol) injected groups ( $p < 0.01$ ). Injection of the nesfatin-1 (50 pmol) caused a significant decrease in the MDA levels of the cerebrum compared to the control group ( $p < 0.05$ ).

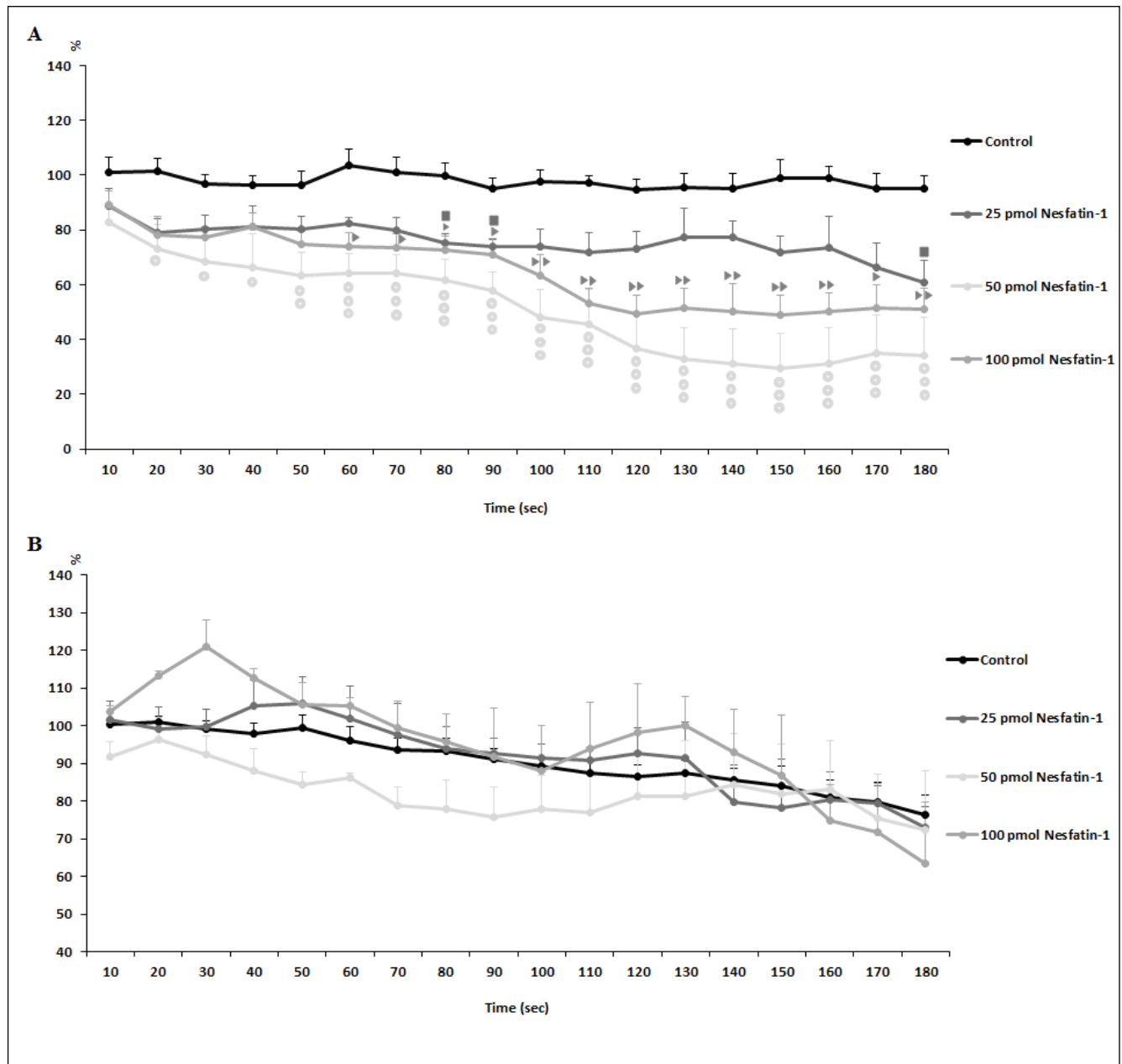


Fig. 2. (A) The effects of i.c.v. administration of nesfatin-1 doses on the mean spike frequency of penicillin-induced epileptiform activity. (B) The effects of i.c.v. administration of nesfatin-1 doses on the mean spike amplitude of penicillin-induced epileptiform activity. Nesfatin-1, at doses of 25, 50 and 100 pmol significantly decreased the mean spike frequency of penicillin-induced epileptiform activity without changing the amplitude. Nesfatin-1, at doses of 12.5 and 200 pmol, did not influence the mean frequency and amplitude of epileptiform activity. Nesfatin-1 (25 pmol) different from control group ■  $p < 0.05$ . Nesfatin-1, (50 pmol) different from control group ★  $p < 0.05$ , ★★  $p < 0.01$ , ★★★  $p < 0.001$ . Nesfatin-1, (100 pmol) different from control group ▶  $p < 0.05$ , ▶▶  $p < 0.01$ , ▶▶▶  $p < 0.001$ . Values are given as mean  $\pm$  standard error.

## DISCUSSION

Our data suggests that nesfatin-1 is an anticonvulsant agent. It was determined that the CRF2 receptor antagonist Astressin 2B, which antagonizes the effect

of nesfatin-1 in reducing food intake, has no effect on epileptic activity. Additionally, nesfatin-1 plays a neuroprotective role in oxidative stress.

Nesfatin-1 modulates neuronal circuits and has both a hyperpolarizing and depolarizing effect on paraven-

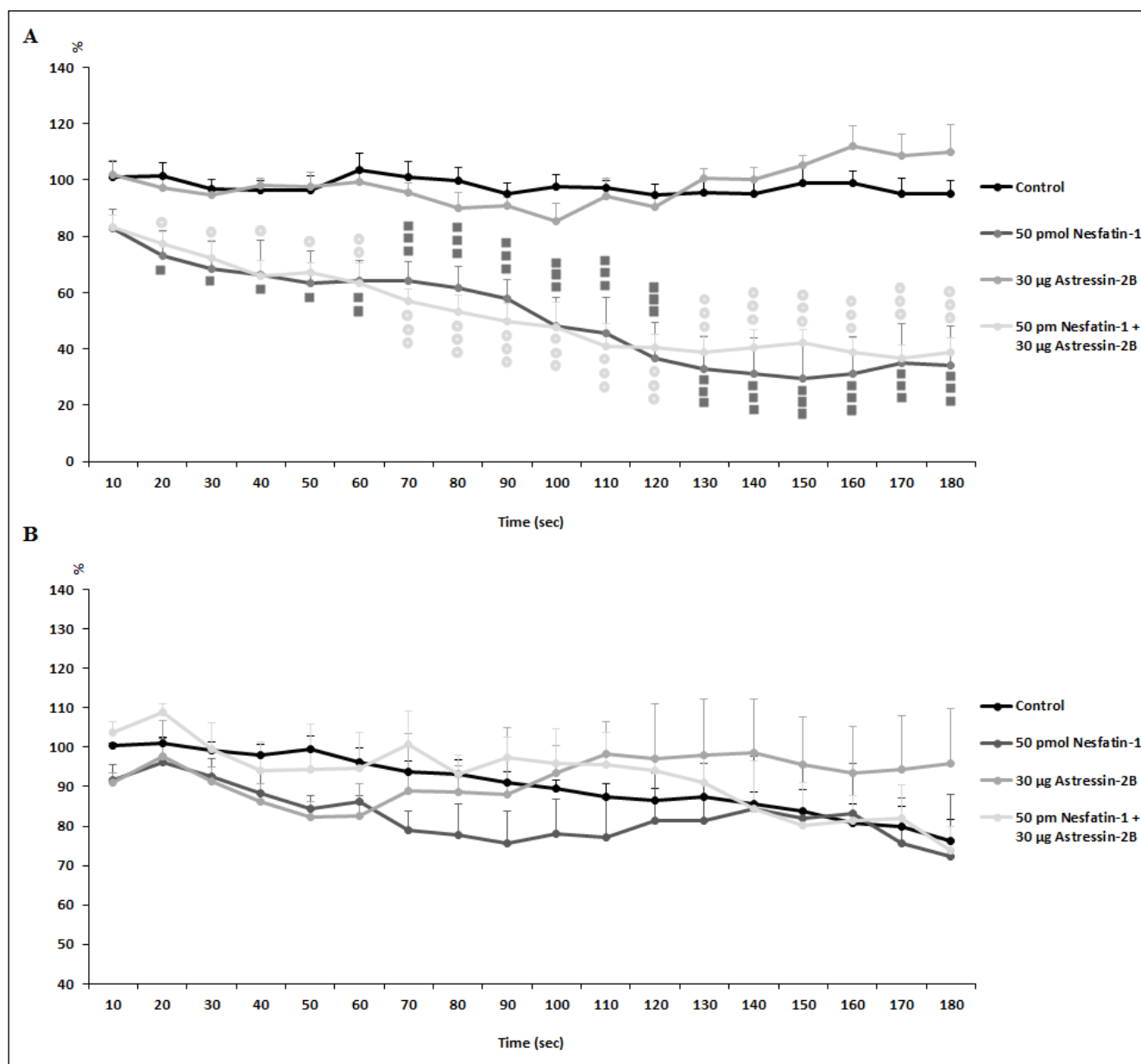


Fig. 3. (A) The effects of administration of astressin 2B (30 µg), nesfatin-1 (50 pmol) and interaction group (nesfatin-1 (50 pmol) and Astressin 2B (30 µg)) on the mean spike frequency of penicillin-induced epileptiform activity. (B) The effects of administration of astressin 2B (30 µg), nesfatin-1 (50 pmol) and interaction group (nesfatin-1 (50 pmol) and astressin 2B (30 µg)) on the mean spike amplitude of penicillin-induced epileptiform activity. Astressin 2B (30 µg), did not affect spike frequency and amplitude of penicillin-induced epileptiform activity. Nesfatin-1 (50 pmol) significantly decreased the mean spike frequency of penicillin-induced epileptiform activity without changing the amplitude. Astressin 2B (30 µg) did not change the effect of nesfatin-1 (50 pmol). Nesfatin-1 (50 pmol) different from control group ■  $p < 0.05$ , ■■  $p < 0.01$ , ■■■  $p < 0.001$ . Nesfatin-1 (50 pmol) + Astressin 2B (30 µg) different from control group ★  $p < 0.05$ , ★★  $p < 0.01$ , ★★★  $p < 0.001$ . Values are given as mean  $\pm$  standard error.

tricular nucleus neurons (Rupp and Stengel, 2022). There are studies in the literature showing that nesfatin-1 may be anti/pro-convulsant agent or that it has no effect on epileptic activity. In response to nesfatin-1 exposure, hyperpolarization in neuropeptide Y neurons, an anticonvulsant (Price et al., 2008; Kovac and

Walker, 2013), and increased intracellular  $\text{Ca}^{2+}$  concentration in cultured rat hypothalamic neurons (Brailoiu et al., 2007) are findings that suggest nesfatin-1 may act as a proconvulsant agent. Additionally, it has been suggested that nesfatin-1 is related to the glutaminergic system and may trigger neuronal activation (Yurtseven

Table 1. The levels of superoxide dismutase (SOD  $\mu\text{M}$ ), glutathione (GSH  $\mu\text{M}$ ), glutathione peroxidase (GPx nmol/min/ml), and glutathione reductase (GR nmol/min/ml) and malondialdehyde (MDA  $\mu\text{M}$ ) in the cerebrum.

Groups	SOD	GSH	GPx	GR	MDA
Penicillin Control	5.03 $\pm$ 0.53	41.69 $\pm$ 0.83	1.96 $\pm$ 0.19	2.54 $\pm$ 0.33	0.96 $\pm$ 0.07
Nesfatin-1 (25 pmol)	5.01 $\pm$ 0.25	42.57 $\pm$ 1.02	2.99 $\pm$ 0.29*	3.96 $\pm$ 0.17*	0.80 $\pm$ 0.02
Nesfatin-1 (50 pmol)	5.46 $\pm$ 0.22	41.90 $\pm$ 1.57	3.62 $\pm$ 0.19**	4.24 $\pm$ 0.25**	0.74 $\pm$ 0.02*
Nesfatin-1 (100 pmol)	5.10 $\pm$ 0.44	43.93 $\pm$ 0.08	3.57 $\pm$ 0.29**	4.04 $\pm$ 0.19**	0.80 $\pm$ 0.07

Versus penicillin-injected group \* $p < 0.05$ , \*\* $p < 0.01$ .

et al., 2020; Kocoglu et al., 2021). Furthermore, nesfatin-1 (100 and 300 pmol) both causes epileptic activity and increases penicillin-induced epileptic activity in rats (Erken et al., 2015). However, Erken et al.'s (2015) study differs from our work in many ways, such as the weight of the experimental animals, the anesthetic substance used, the intracortical application of nesfatin-1, the total recording time of the epileptic activity (120 min), the time points at which the epileptic activity was assessed. On the other hand, nesfatin-1 (i.p.) administration had no anti/pro-convulsant effect on PTZ (80 mg/kg) induced acute seizures in mice (Erkek, 2021). According to our findings, nesfatin-1 shows an anticonvulsant effect. Nesfatin-1 hyperpolarizes dopamine, but not gamma aminobutyric acid (GABA) in the ventral tegmental area (Dore et al., 2020). Furthermore, NUCB2/nesfatin-1 was found to decrease the excitability of dopaminergic neurons in the presence of ionotropic glutamate and GABA receptor antagonists (Li et al., 2014). These studies suggest that nesfatin-1 may have an anticonvulsant effect. Additionally, nesfatin was found to reduce the epileptic seizure score in epilepsy induced by PTZ in rats (Tamer et al., 2022).

CRF2 antagonist astressin 2B abolished (i.c.v.) nesfatin-1's anorexigenic action (Stengel et al., 2009). In a different study, astressin 2B inhibited renal sympathetic and hypertensive responses to i.c.v. injection of nesfatin-1 (Tanida and Mori, 2011). In our study, we used the CRF2 antagonist astressin 2B in order to better understand the effect of nesfatin-1 on seizure activity. Nesfatin-1 increased cytosolic  $\text{Ca}^{2+}$  concentration in the CRF-immunoreactive neurons and increased plasma ACTH and corticosterone levels (Yoshida et al., 2010). In our study, only astressin 2B did not affect the frequency and amplitude of epileptic activity. In addition, it showed a similar effect on the 50 pmol doses of nesfatin-1.

Oxidative stress and mitochondrial dysfunction can produce epilepsy and not be merely a consequence of seizures (Pearson-Smith and Patel., 2017). There are conflicting results regarding the levels of

oxidative stress markers in epilepsy. Injection of penicillin caused a significant increase in the MDA levels and significantly decreased GPx and GR levels, but the penicillin injection did not alter SOD and GSH levels in the cerebrum (Arslan et al., 2019). Decreased expression of MDA, and increased expression of SOD and GPx alleviates oxidative stress (Liu et al., 2019a). PTZ kindling also increased the MDA content and reduced the SOD activities in the hippocampus (Liu et al., 2019b). Memantine, which is considered an anticonvulsant, reduced MDA, GSH, and GR levels in the cerebrum in WAG/Rij rats absence epilepsy model (Doğan et al., 2020). In this study, nesfatin-1 had an effect in terms of increasing GPx and GR and decreasing MDA, but it was ineffective on SOD and GSH in the cerebrum. Arabacı Tamer et al.'s (2022) study showed that nesfatin-1 suppressed the levels of MDA levels but elevated GSH levels in the epileptic experimental model of PTZ. nesfatin-1 has an antioxidant effect by decreasing MDA levels and increasing SOD and GSH levels (Xu and Chen, 2020). There are study showing that nesfatin-1 has an improving effect in neurological conditions other than epilepsy, such as ischemic stroke (Erfani et al., 2019). In addition, there is lower nesfatin-1, and to higher total oxidant status levels, in patients with Parkinson's disease (Emir et al., 2019).

## CONCLUSIONS

Despite recent advances in nesfatin-1 research, no putative receptor has been identified and the potentially significant functions of nesfatin-1 and its precursor NUCB2 have not yet been studied. Expanding our knowledge of NUCB2/nesfatin-1 should be the main target of future research. In this study, nesfatin-1 had the effect of reducing epileptic activity and this effect was mediated by reducing oxidative stress. With this detailed explanation of the mechanism of nesfatin-1 action, it may thus be used as a protective agent against seizures.



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