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ORIGINAL ARTICLE

The potential effects of anticonvulsant drugs on neuropeptides and neurotrophins in pentylenetetrazol kindled seizures in the rat

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ABSTRACT

Purpose: Neuropeptides and neurotrophic factors are thought to be involved in epileptogenesis. This study aims to investigate the potential effects of anticonvulsant drugs on neuropeptides (galanin and neuropeptide Y) and neurotrophic factors (BDNF and NGF) in pentylenetetrazol (PTZ)-kindled seizures in the rat.

Methods: Forty-eight adult male Sprague–Dawley rats were included in the study. The animals were divided into 8 groups of six rats. Group 1 was defined as naïve control, and received no medication. Group 2 (PTZ + saline) was treated with sub-convulsive doses of PTZ (35 mg/kg) and saline i.p. for 14 days. For anticonvulsant treatments, Groups 3–8 were treated with 200 mg/kg levetiracetam (PTZ + LEV), 1 mg/kg midazolam (PTZ + MDZ), 80 mg/kg phenytoin (PTZ + PHT), 80 mg/kg topiramate (PTZ + TPR), 40 mg/kg lamotrigine (PTZ + LMT) and 50 mg/kg sodium valproate (PTZ + SV), respectively. All anticonvulsant drugs were injected 30 min prior to PTZ injection throughout 14 days. Following treatment period, behavioral, biochemical and immunohistochemical studies were performed.

Results: PTZ + saline group revealed significantly decreased galanin, NPY, BDNF and NGF levels compared to control. PTZ + MDZ group had significantly increased galanin, BDNF and NGF levels compared to saline group. Also, PTZ + LEV group showed increased BDNF levels. PTZ + saline group revealed significantly lower neuron count and higher GFAP (+) cells in hippocampal CA1–CA3 regions. All anticonvulsants significantly reduced hippocampal astrogliosis whereas only midazolam, levetiracetam, sodium valproate and lamotrigine prevented neuronal loss.

Conclusion: Our results suggested that anticonvulsant drugs may reduce the severity of seizures, and exert neuroprotective effects by altering the expression of neuropeptides and neurotrophins in the epileptogenic hippocampus.

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1. Introduction

Epilepsy is considered to be a neuronal network disorder characterized by recurrent seizures. Worldwide, up to 3% of the general population have epilepsy at some point in their lives [1], and in the United States, it is estimated that nearly two million people have epilepsy. While we've made tremendous advances in our understanding of epilepsy over the past 50 years, many instances of epilepsy remain idiopathic with unclear pathophysiology [2]. Likewise, there continue to be great challenges in the treatment of epilepsy. In spite of the growing diversity of antiepileptic drugs, approximately 30% of epilepsy patients continue to experience seizures despite optimal antiepileptic therapy [3], and thus they are deemed to have resistant or refractory disease. The number of drugs marketed to treat epilepsy has been rapidly growing with most antiepileptic drugs targeting either sodium channels or GABA receptors. Unfortunately, despite the burgeoning of new antiepileptic drugs, one-third of patients with epilepsy continues to have seizures and their epilepsies are resistant to all current available treatments [4]. In addition, the classic antiepileptic drugs which typically target ion channels or ionotropic receptors have substantial side effects which are causally linked to their mechanism of action.

Epileptic seizures are caused by a disturbance of the balance between inhibition and excitation in the brain, and neuropeptides, such as galanin and neuropeptide

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Y (NPY) are thought to be involved in epileptogenesis [5–8]. Neuropeptides have been explored as potential alternatives to classic antiepileptic drugs [9]. Unlike classical neurotransmitters, neuropeptides act through indirect modulation of neurotransmitter release or ion channel/receptor function. Neuropeptides are powerful modulators of the inhibitory neurotransmitter GABA, and the excitatory neurotransmitter glutamate either by modifying their release or by regulating their effects at the receptor level. Neuropeptides exert both pre- and postsynaptic actions on excitatory and inhibitory transmission [10]. A long list of neuropeptides has been explored for the treatment of epilepsy. Of those the most promising candidates with multiple reports corroborating their direct antiepileptic and antiepileptogenic action include neuropeptide Y (NPY), galanin, dynorphin, somatostatin and ghrelin. In contrast, substance P and the tachykinens seem to have a pro-epileptic effect. Neuropeptides and their receptors are interesting targets for the development of novel anticonvulsant drugs [5–8].

Galanin and neuropeptide-Y (NPY) have been reported to act as potent endogenous anticonvulsant peptides [5, 6, 11–13]. NPY, a 36-amino acid peptide, is one of the most studied neuropeptides in the epilepsy. NPY is predominantly expressed in GABAergic interneurons in the brain, including the hippocampus. NPY can act through five different receptors Y1–Y5, but mainly shows its effects by binding to Y1, Y2 and Y5 [14]. NPY induces strong, but transient suppression of spikes and seizures, while galanin is more profound in seizure suppression [15]. The neuropeptide galanin and its G protein-coupled receptors (GAL 1–3) are also widely distributed in the nervous system [7, 16]. Galanin exerts its anticonvulsant effect via presynaptic inhibition of excitatory glutamatergic transmission in the hippocampus [17]. Recently, galanin receptors are considered as an interesting target for anticonvulsive therapy.

Experimental studies have revealed that the activation of neurotrophins, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) promotes epileptogenesis [18–22]. BDNF, a small dimeric protein, mainly exists in hippocampus, amygdala, cortex and cerebellum. It is expressed by both neurons and glia, and involved in survival, differentiation and regeneration of neurons through binding its receptor, TrkB. BDNF dysregulation has been implicated in various neurologic and psychiatric diseases. BDNF is known to regulate the expression of NPY, which modulates seizure activity [8, 19, 23]. This evidence suggests that the effects of BDNF on epileptogenesis may be mediated by NPY. However, there are still several controversial viewpoints about the role of BDNF in epileptogenesis.

PTZ-kindling is one of the most important epilepsy models used for understanding the epileptogenic process, and molecules that affect this process [24–27]. This model is also used for investigating neurochemical and long-term structural changes in the epileptic network of specific brain area [28]. It has been demonstrated that kindling results in mossy fiber sprouting, alterations in neurotrophins, and their receptors [29, 30].

The modulatory effects of neuropeptides and neurotrophins are widely studied in several animal models of epilepsy. However, there are limited studies which examine the effects of anticonvulsant drugs on neuropeptide and neurotrophin levels in human and animal models [31–34]. The aim of the present study was to investigate the effects of a collection of conventional and new anticonvulsant drugs on brain galanin, NPY, BDNF and NGF levels, and hippocampal integrity by using PTZ-kindling model in rats.

2. Materials and methods

2.1. Animals and ethical statement

Forty-eight adult male Sprague–Dawley rats (200–250 g) were included in the study. The rats were kept on a 12-hour light/dark cycle (light from 07.00 to 19.00), in quiet rooms, with 22–24 °C ambient temperature. They were fed by standard laboratory food and tap water ad libitum. All experimental procedures employed in the present study were approved by Ege University Animal Ethics Committee (authorization number: 2013-16).

2.2. Study design

Figure 1 summarizes the experimental design used in the study. A protocol for producing kindling in rats was performed according to the literature [35, 36]. The animals were divided into 8 groups of six rats ($n = 6$). Group 1 was defined as naïve control, and given no medication. Group 2 was assigned to placebo group (PTZ + saline), and were administered with PTZ (35 mg/kg, i.p.) and physiological saline solution (i.p.) at a dose of 1 ml/kg/day. Sub-convulsive doses of PTZ (35 mg/kg) (Sigma-Aldrich, St Louis, MO) were injected once-daily for 14 days. PTZ was freshly dissolved in physiological saline before administering injections. For the anticonvulsant treatments, Groups 3–8 were treated with 200 mg/kg levetiracetam (PTZ + LEV; UCP Pharma A.S., Istanbul, Turkey), 1 mg/kg midazolam (PTZ + MDZ; Pharmada A.S., Istanbul, Turkey), 80 mg/kg phenytoin (PTZ + PHT; Pfizer Ltd., Istanbul, Turkey),

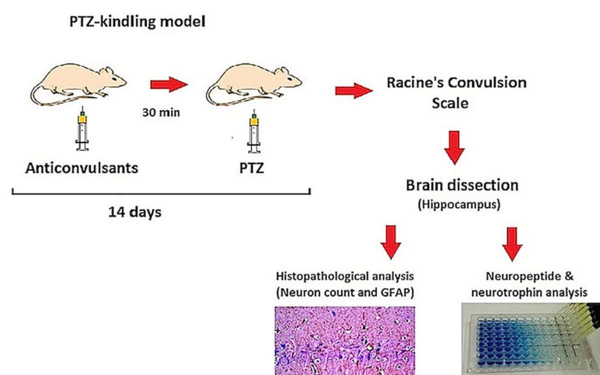

Figure 1. Schematic diagram of the study design.

80 mg/kg topiramate (PTZ + TPR; Johnson & Johnson Ltd., Istanbul, Turkey), 40 mg/kg lamotrigine (PTZ + LMT; Glaxo Smith Kline Ltd., Istanbul, Turkey) and 50 mg/kg sodium valproate (PTZ + SV; Sanofi Ltd., Istanbul, Turkey), respectively. All anticonvulsant drugs were injected intraperitoneally 30 min prior to PTZ injection throughout 14 days. The doses and the duration of therapy were determined according to the previous studies [21, 36–38]. The rats were housed in Plexiglass cages ($50 \times 50 \times 50$) after the PTZ injection, and the occurrence of seizures was observed for 30 min, as previously described [39]. Racine's Convulsion Scale (RCS) was used to evaluate the seizures as follows: 0 = no convulsion; 1 = twitching of vibrissae and pinnae; 2 = motor arrest with more pronounced twitching; 3 = motor arrest with generalized myoclonic jerks; 4 = tonic clonic seizure while the animal remained on its feed; 5 = tonic-clonic seizure with loss of the righting reflex; and 6 = lethal seizure. After completion of PTZ and drug treatments, the animals were euthanized, and the brains were removed for histopathological and biochemical evaluations.

2.3. Tissue collection

Following decapitation, brains were rapidly removed, and divided into two hemispheres. The right-side brain tissues were used for biochemical evaluation, and the remaining left-side brain tissues were used for histology. Hippocampal tissues were stored at −20 °C until to biochemical evaluation. The second half of each brain was fixed in 10% formaldehyde in 0.1 M phosphate buffered saline for histological and immunohistochemical examination [40].

2.4. Biochemical evaluation

For biochemical analysis, frozen hippocampi were homogenized with a glass homogenizer in 5 volume of phosphate buffered saline (pH 7.4), and centrifuged at $5000 \times g$ for 15 min. The supernatant was then collected, and the total protein concentration in the tissue homogenates was determined according to Bradford's method [41]. The galanin, NPY, BDNF and NGF levels were measured by using commercially available enzyme-linked immunosorbent assay kits (Shanghai Sunred Biological Technology Co., Ltd.) specific for rats. Samples from each animal were determined in duplicate according to the manufacturer's guidelines. Absorbances were measured by a microtiter plate reader (MultiscanGo, Thermo Fisher Scientific Laboratory Equipment, New Hampshire). The detection ranges for each ELISA were 5–1500 ng/L, 3–900 ng/L, 0.04–10 ng/mL and 0.3–90 ng/mL, respectively. Intraassay and interassay coefficients of variation were less than 10% in each determination. The results were expressed as picograms (for galanin and NPY) and nanograms (for BDNF and NGF) per milligram of protein.

2.5. Histopathological evaluation

For histological assessment, brain tissues were fixed for 3 days in 10% formaldehyde in 0.1 phosphate buffered saline. Then, they were moved into 30% sucrose and stored at 4 °C until infiltration was complete. Ten-micrometer-thick coronal brain sections at the level of the dorsal hippocampus (plates 21, 23 and 25) were prepared according to a stereotaxic atlas [42]. The hippocampal sections were stained with the Nissl (cresyl violet) staining techniques, for visualizing cytoarchitecture of the hippocampal cell layers and neuron count. Histological evaluation was accomplished in three to four sections per hippocampus ($n = 6$) for each rat ($n = 6$) by an image analysis system (Image-Pro Express 1.4.5, Media Cybernetics, Inc. USA), in a blinded manner, as described previously [40].

For GFAP immunohistochemistry, brain sections were incubated with H_2O_2 (10%) for 30 min to eliminate endogenous peroxidase activity and blocked with 10% normal goat serum (Invitrogen) for 1 h at room temperature. Subsequently, sections were incubated in primary antibodies against GFAP (Abcam, Cambridge, MA; 1/1000) for 24 h at 4 °C. Antibody detection was carried out with the Histostain-Plus Bulk kit (Invitrogen) against rabbit IgG, and 3,3-diaminobenzidine was used for visualization of the final product. All sections were washed in PBS and photographed with an Olympus C-5050 digital camera mounted on an Olympus BX51 microscope. GFAP immunoexpression was determined by counting GFAP (+) cells at 40× magnification in randomized sections (3–4) for each rat ($n = 6$) in a blinded fashion [40].

2.6. Statistical analysis

Results are presented as mean ± standard error of mean (SEM). The Shapiro–Wilk test was used to see parametric and non-parametric distribution. All data were analyzed by one-way ANOVA followed by Tukey's test. p Values less than 0.05 were considered significant. Statistical analysis was performed using SPSS version 18.0 for Windows.

Table 1. Behavioral seizure scores.

Groups	Convulsion stage
Naïve control ($n = 6$)	0
PTZ + saline ($n = 6$)	5.6 ± 0.21
PTZ + LEV ($n = 6$)	3.6 ± 0.22*
PTZ + MDZ ($n = 6$)	1.8 ± 0.34***
PTZ + PHT ($n = 6$)	3.8 ± 0.18*
PTZ + TPR ($n = 6$)	3.2 ± 0.34**
PTZ + LMT ($n = 6$)	2.8 ± 0.34**
PTZ + SV ($n = 6$)	3 ± 0.41**

Results are presented as mean ± standard error of mean (SEM).
*$p < 0.005$ (comparing with PTZ + saline).
**$p < 0.0001$ (comparing with PTZ + saline).
***$p < 0.0005$ (comparing with PTZ + saline).

3. Results

3.1. Convulsion scores

All animals were monitored daily for behavior and health conditions. There was no mortality in the study groups. Racine's Convulsion Scale (RCS) was used to evaluate the seizures. Table 1 depicts the convulsion scores of all groups obtained at 14th day of the study. Comparing the convulsion scores among the groups indicated the severity of seizures after the injection of PTZ. All anticonvulsant drug received groups showed significantly decreased scores compared to the saline group. The most significant effect was observed in PTZ + midazolam group ($p < 0.0005$).

3.2. Effects of anticonvulsant drugs on neuropeptide and neurotrophin levels

Figure 2 presents the alterations in hippocampal galanin, NPY, BDNF and NGF levels in the study groups. Galanin levels were significantly decreased in the saline, phenytoin, topiramate and sodium valproate

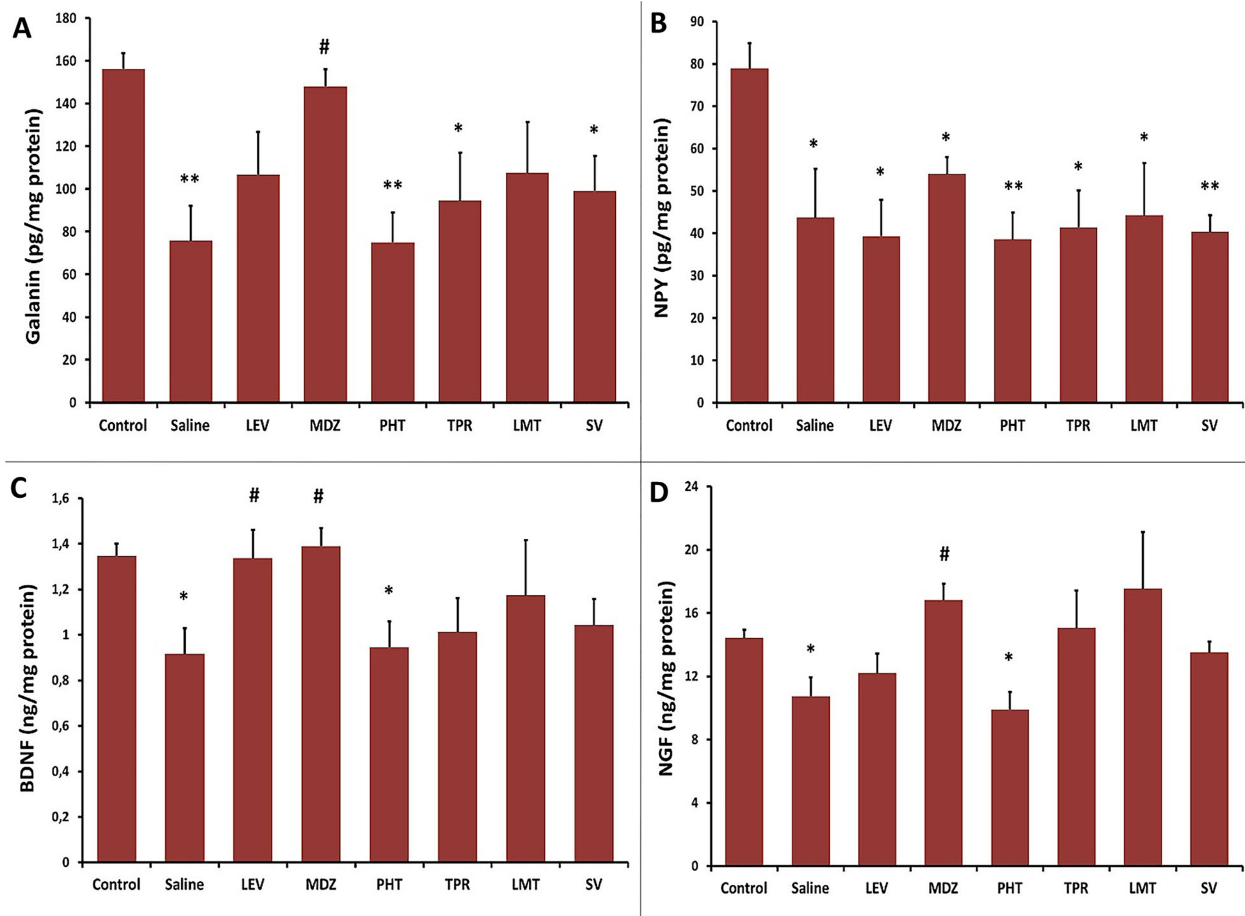

Figure 2. The effects of anticonvulsants on brain neuropeptides and neurotrophins in PTZ-kindled rats. Results are expressed as mean ± SEM. *$p < 0.05$ and **$p < 0.005$ different from control, #$p < 0.05$ different from saline.

administered groups in comparison with control group ($p < 0.005$, $p < 0.005$, $p < 0.05$ and $p < 0.05$, respectively), while no significant alteration was observed in lamotrigine and levetiracetam administered groups ($p > 0.05$). As well, in the PTZ + midazolam group, galanin levels were greatly enhanced in comparison with that in the saline-treated group ($p < 0.01$, Figure 2(A)). NPY levels were significantly decreased in all study groups compared to control ($p < 0.05$ and $p < 0.005$, Figure 2(B)). BDNF levels were significantly decreased in the saline and phenytoin groups compared to control ($p < 0.05$) Levetiracetam and midazolam-treated groups displayed significantly higher BDNF levels than those in saline-treated group ($p < 0.05$, Figure 2(C)). Similarly, NGF levels were significantly decreased in the saline and phenytoin groups compared to control ($p < 0.05$) whereas midazolam group had significantly higher levels of NGF compared to saline group ($p < 0.05$, Figure 2(D)). However, no significant differences were observed in other anticonvulsant-treated groups in terms of BDNF and NGF levels.

3.3. Effects of anticonvulsant drugs on hippocampal integrity

In order to explore the potential effects of anticonvulsant drugs on hippocampal integrity, neuron count and GFAP immunohistochemistry were performed in the hippocampus. Figures 3 and 4 depict the histopathological alterations in hippocampal CA1 and CA3 regions in the study groups, respectively. Neuron counts in the CA1–CA3 regions of PTZ + saline group showed a marked decrease compared to the control group ($p < 0.0001$, Table 2), thus indicating a significant damage in hippocampal neurons. However, midazolam, levetiracetam, lamotrigine and sodium valproate-received groups had significantly higher neuron number in comparison with that in the saline-treated group ($p < 0.05$, Table 2). No significant alterations were found in the phenytoin and topiramate groups.

Examination of hippocampal CA1 and CA3 regions revealed significantly higher GFAP immunoreactivity in the PTZ + saline group compared to the control group ($p < 0.0001$, Table 2), that strongly confirmed gliosis. However, all anticonvulsant-treated groups had significantly decreased GFAP (+) cells in comparison with the PTZ + saline group ($p < 0.05$ and $p < 0.005$, Table 2).

4. Discussion

Neuropeptides and neurotrophins are thought to have important roles in the pathogenesis of epilepsy and be involved in neuronal repair of this network. Altered expression of neuropeptides and neurotrophins has been reported in the clinical studies and experimental epilepsy models. Aberrant neuropeptide levels have been found in plasma, cerebrospinal fluid (CSF) and resected tissues from epilepsy patients and in different animal seizure models [43]. Some of these neuropeptides have been implicated in the regulation of seizure susceptibility. In fact, many endogenous neuropeptides display anticonvulsive effects in animal models of epilepsy. Hence, these neuropeptides and their receptors are attractive targets for the development of new antiepileptic drugs [43]. Other neuropeptides show pro-convulsive effects, which makes their receptors also interesting targets for suppressing seizures using, receptor specific antagonists. As neuropeptide expression is significantly altered in specific epileptic conditions, neuropeptides might also be considered as biomarkers. Since the treatment of seizures depends on an accurate diagnosis, making sure that a person has epilepsy and knowing what kind is a critical first step. According to these needs, we aimed to investigate the effects of a collection of conventional and new anticonvulsant drugs on brain galanin, NPY, BDNF and NGF levels, and hippocampal integrity. In the present study, we demonstrated significantly decreased levels of brain galanin, NPY, BDNF and NGF levels in the PTZ-kindled rats. Also, we found significant alterations in brain neuropeptide and neurotrophin levels in the anticonvulsant-administered rats prior to kindling procedure. Among the investigated anticonvulsant drugs, midazolam provided considerably elevated levels of BDNF, NGF and galanin compared to saline-treated group. The impairment of the $GABA_A$ receptor-dependent maturation of neuronal circuits caused by PTZ can lead to permanent changes in neuronal network that may result in enhanced seizure susceptibility. Increased galanin levels in hippocampal tissue maybe due to anti-seizure effect of midazolam in neuronal networks. The first compelling observations pointing to a role of galanin in epilepsy were made in animal models of status epilepticus. Mazarati and colleagues found that rats subjected to continuous seizure activity such as is seen in sustained status epilepticus (SSSE) exhibit profound and prolonged galanin depletion within the hippocampus for at least a week after SSSE [5]. They also showed injection of galanin into the hilus of the hippocampus reduced the duration of SSSE. Besides, levetiracetam administration caused increase in BDNF levels in PTZ-kindled rats. The histopathological evaluations of the hippocampal CA1-CA3 areas also showed that neuronal loss and astrogliosis

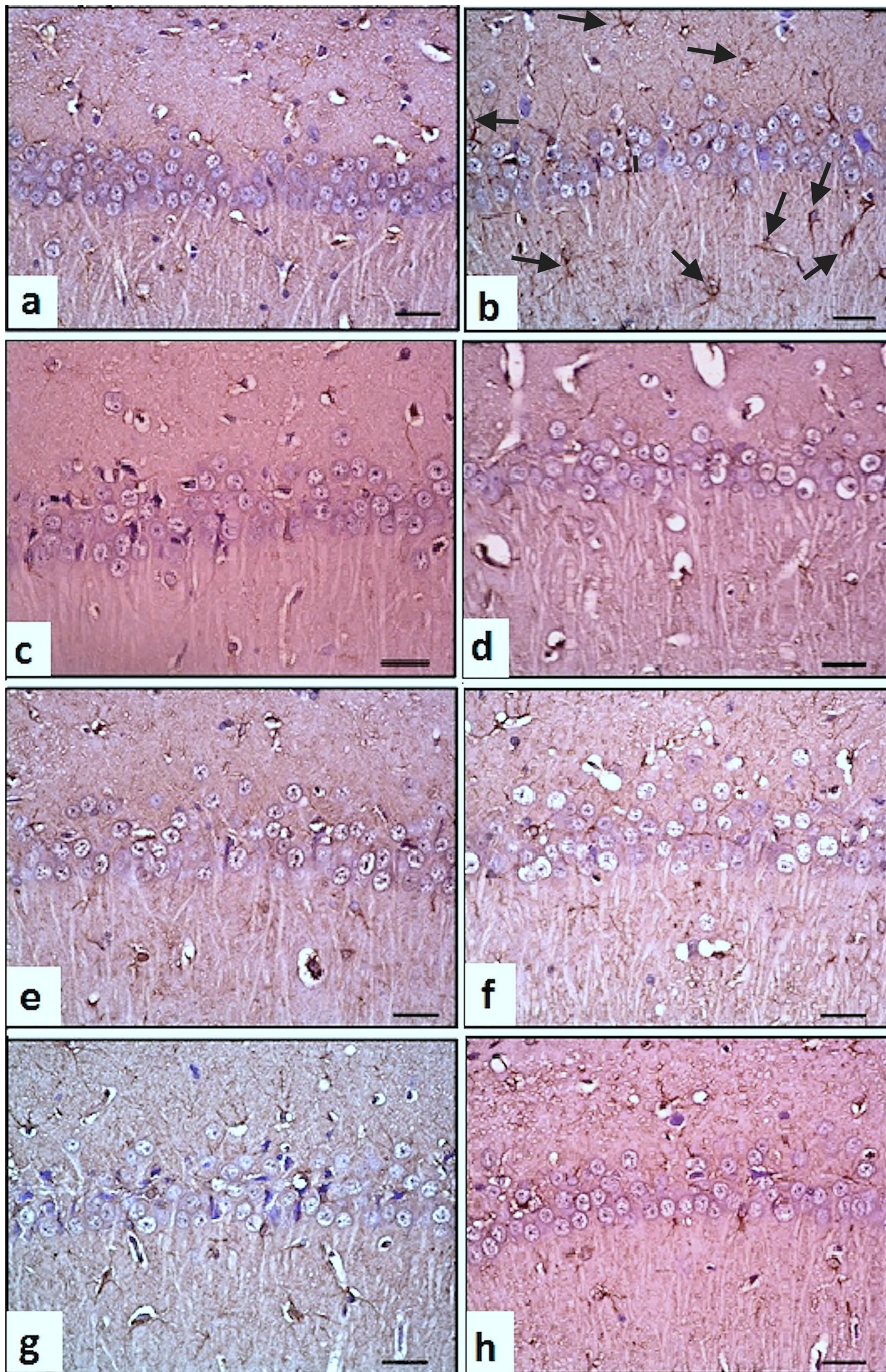

Figure 3. The effects of anticonvulsants on hippocampal CA1 neurons and GFAP (+) astroglial cells in PTZ-kindled rats. Arrows indicate GFAP (+) cells. a) Control, b) Saline, c) LEV, d) MDZ, e) PHT, f) TPR, g) LMT, h) SV. Bars= 100 μm, $n = 6$.

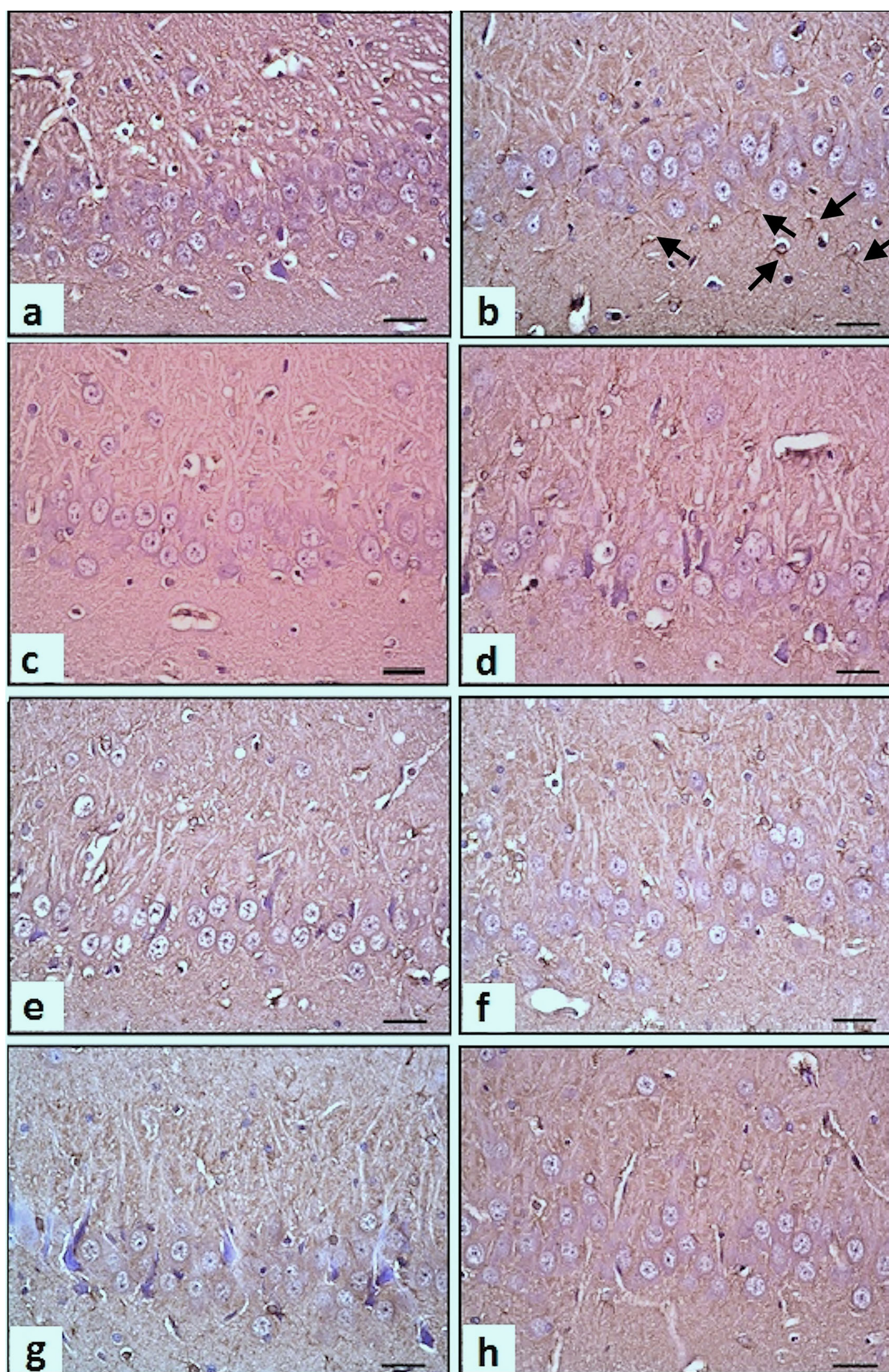

Figure 4. The effects of anticonvulsants on hippocampal CA3 neurons and GFAP (+) astroglial cells in PTZ-kindled rats. a) Control, b) Saline, c) LEV, d) MDZ, e) PHT, f) TPR, g) LMT, h) SV. Bars = 100 μm, $n = 6$.

Table 2. The effects of anticonvulsant drugs on neuron count and GFAP immunoreactivity in PTZ-kindled rats.

Groups	CA1 neuron count	CA3 neuron count	CA1 GFAP (+) cell count	CA3 GFAP (+) cell count
Naïve control ($n = 6$)	66.83 ± 2.02	43.2 ± 3.28	15.67 ± 1.12	10.83 ± 0.5
PTZ + saline ($n = 6$)	46.50 ± 2.0*	30.3 ± 1.8*	30.1 ± 1.3*	20.5 ± 1.48*
PTZ + LEV ($n = 6$)	52.33 ± 1.93#	39.2 ± 2.50#	12.83 ± 1.25##	10.5 ± 0.8##
PTZ + MDZ ($n = 6$)	60.83 ± 2.23#	37.5 ± 0.9#	11.01 ± 0.73##	13.8 ± 0.9#
PTZ + PHT ($n = 6$)	48.17 ± 3.21	34.5 ± 1.5	17.67 ± 1.02#	13.50 ± 0.76#
PTZ + TPR ($n = 6$)	48.83 ± 1.89	33.8 ± 1.7	20.17 ± 1.25#	12.7 ± 1.05##
PTZ + LMT ($n = 6$)	53.50 ± 2.05#	36.17 ± 1.96#	21.33 ± 1.05#	15.17 ± 0.95#
PTZ + SV ($n = 6$)	58.33 ± 1.26#	37.5 ± 2.8#	12.17 ± 0.95##	13.2 ± 1.3#

Results are presented as mean ± standard error of mean (SEM).
*$p < 0.0001$ (comparing with naïve control).
#$p < 0.05$ (comparing with PTZ + saline).
##$p < 0.005$ (comparing with PTZ + saline).

were reduced by midazolam and levetiracetam. Our results suggested that these two anticonvulsant drugs may have beneficial effects in epileptogenesis by modulating of neuropeptides and neurotrophins, and preserving neuronal viability in the hippocampus.

Neurotrophins such as BDNF and NGF have been reported to play opposite, pro- and/or anti-epileptic effects in experimental models [44–49]. Although facilitation of excitatory transmission is a pronounced effect of BDNF, it has been reported that BDNF could potentiate GABAergic inhibition in human epileptic brain [46]. It is probable that BDNF diminishes the overall activity of hippocampus. As revealed previously, BDNF increases the density of inhibitory synapses in hippocampal slices [47], and amplify the body size of GABAergic neurons [48]. Intriguingly, increased expression of BDNF has been revealed in hippocampal specimens from patients with temporal lobe epilepsy and kindling animal models [19], while serum BDNF levels have been found to be diminished in patients with epileptic and psychogenic nonepileptic seizures [22]. It is also known that BDNF regulates the expression of NPY, which modulates seizure activity [8, 19, 23]. NPY may not only modulate excitability but also induces neurogenesis. Increased expression of NPY in the dentate gyrus, CA1 and CA3 regions of the epileptic hippocampus has been reported in other animal studies [8, 23, 50].

Preliminary reports have explored the effects of anticonvulsant drugs on neuropeptides and neurotrophins in animal models and epileptic children [32–34, 50]. In the present study, in addition to alterations in neuropeptides and neurotrophins in PTZ-kindled rats, we observed that pre-treatment of the rats with levetiracetam markedly delayed the progression of kindling, increased BDNF levels, preserved neuronal viability and reduced hippocampal gliosis. Furthermore, we detected a tendency to increase in NPY levels in midazolam-treated group compared to saline-treated group but with no statistical significance. NPY acts as an endogenous anticonvulsant. In keeping with the peptide's distribution, NPY-receptor expression is high in brain areas involved in the initiation and propagation of temporal lobe seizures, such as the hippocampus [51]. NPY is considered an endogenous anticonvulsant and studies in the hippocampus in vitro have shown that NPY inhibits glutamatergic synaptic transmission in areas CA1 and CA3 [52]. The elevation of NPY levels in the midazolam group may be the result of increased BDNF levels in the epileptic hippocampus.

The main molecular mechanisms that are responsible for the action of anticonvulsant drugs can be related to inhibition of neuronal excitatory (i) Na^{+} channels; (ii) low voltage-activated (T-type) Ca^{2+} channels or (iii) facilitation of inhibitory GABAergic neurotransmission. Among the investigated drugs in the present study, anticonvulsant action of midazolam is mainly via GABAergic neurotransmission. On the other hand, the antiepileptic effect of levetiracetam is mediated through binding to synaptic vesicle glycoprotein 2A (SV2A), a protein localized in the membrane of synaptic vesicles, and suppressing Ca^{2+} mobilization from the endoplasmic reticulum. Levetiracetam also affects GABA-receptor mediated currents, and reverses the inhibitory effects of GABA and glycine receptor modulators [53]. Besides, neuroprotective effects of levetiracetam have been reported in several experimental models such as hypoxic ischemic brain injury [37], traumatic brain injury [54] and Parkinson's disease [55]. In the present study, in accordance with the previous reports, levetiracetam treatment considerably prevented the neuronal loss and reactive astrogliosis in the epileptic hippocampus.

Interestingly, there was no significant difference between the groups treated with phenytoin, sodium valproate, lamotrigine, topiramate and saline-treated group in terms of neuropeptide and neurotrophin levels. However, among these anticonvulsant drugs, sodium valproate and lamotrigine considerably prevented hippocampal damage by attenuating neuronal

loss and reactive astrogliosis. We, however, were unable to find similar studies of animal models to compare our results. In a previous study conducted by Chen et al, topiramate but not lamotrigine have significantly increased the number of aberrant newborn neurons in the dentate gyrus in a chronic seizure model in rats [56]. On the other hand, in a single dose kainate-induced seizure model, Halbsgut et al. have demonstrated the marked reductions in the number of degenerating neurons within the CA1 subregion, but not in the CA3 subregion of lamotrigine and sodium valproate pre-treated rat pups [57]. More recently, it has been found that both lamotrigine at doses of 25 and 50 mg/kg, and sodium valproate (300 mg/kg) significantly improved epilepsy-induced cognitive impairment and hippocampal neuronal apoptosis in rats with PTZ-kindling model [58]. The discrepancies between the studies might be explained by the different seizure models, age of the animals and different drug administration protocols used in the relevant studies.

This study has some limitations which have to be pointed out. First, we did not get EEG recordings for comparison of the groups in terms of the spike percentage as a reproducible way of quantifying epileptiform activity. Establishing a correlation between electrophysiology and tissue levels of galanin, NPY, BDNF and NGF might have offered additional information about the effects of anticonvulsant drugs on these neuropeptides and neurotrophins. Also, the lack of data about time and dose-dependent effects of the anticonvulsant drugs on neuropeptides and neurotrophins is another limitation of the presented study.

From these data, we concluded that understanding the role of neuropeptides and neurotrophins in epilepsy will lead to development of novel anticonvulsant therapies as well as labeling of some anticonvulsant drugs as antiepileptogenic or neuroprotective agents. However, additional studies are necessary to provide further insight into the physiology of neuropeptides and neurotrophins in the epileptogenesis.

Disclosure statement

No potential conflict of interest was reported by the authors.

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