

Pregabalin, gabapentin and nifedipine in pentylenetetrazole-kindled mice

Ahmed Shata¹ and Abdel-Moneim Hafez²

¹Department of Clinical Pharmacology, Mansoura University, Mansoura, Egypt

²Department of Medical Physiology, Mansoura University, Mansoura, Egypt

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ABSTRACT :

Epilepsy is a big problem facing the society. There are a different modalities of treatment of epilepsy that extend for a long period. Pregabalin is classified as a miscellaneous analgesic and anticonvulsant and is structurally related to gabapentin. Most of antiepileptic drugs can reduce neuronal hyperexcitability by inhibiting ion channels including Ca channels. The current study aimed to evaluate the role of nifedipine in combination with pregabalin and gabapentin in pentylenetetrazole (PTZ)-kindled mice.

Sixty mice were divided into 6 main groups (n=10), positive control group treated by sub-convulsive dose of PTZ (35 mg/kg, i.p.), nifedipine treated group (20 mg/kg), pregabalin treated group (40 mg/kg i.p.), gabapentin treated group (200 mg/kg i.p.), nifedipine + pregabalin group, nifedipine+ gabapentin group. Pain tolerance measurement using right paw pad pressure tolerance was done. Our data indicate that gabapentin, pregabalin and nifedipine significantly decrease seizure activity in comparison to PTZ control mice ($p < 0.05$) and these effects increase more significantly with combination of these drugs ($p < 0.009$, $p < 0.011$). In addition, gabapentin, pregabalin and nifedipine increase significantly pain tolerance in relation PTZ control mice ($p < 0.05$) and coimplementation of both drugs either gabapentin with nifedipine or pregabalin with nifedipine show more synergistic significant effects on pain tolerance ($p < 0.001$).

In conclusion coapplication of these drugs together may lead to decrease of side effects and more control of epilepsy.

Keywords: Kindling, pregabalin, gabapentin, nifedipine, epilepsy, pain.

INTRODUCTION:

Epilepsy is a serious brain disorder. About large number of epileptic patients are not fully controlled with available drugs, also, they have psychiatric and somatic comorbidities, and adverse effects from antiepileptic drugs (Löscher and Schmidt, 2011).

Calcium ion is a regulator of metabolic pathways [Kučák et al., 2004]. The first stage of epileptic neuronal events was evoked by a calcium ion flux into the intracellular space [Caddick et al., 1999]. Its inward current initiate epileptogenic activity in the neuron [de Falco et al., 1992] and these events in neurons were found to be depressed by calcium channel blockers [Hockerman et al., 1997]. The propagation of action potentials along axons transmits information throughout the nervous system. When the presynaptic axon terminal is stimulated by an action potential, there is an influx of Ca^{2+} triggering the release of neurotransmitters that bind to the postsynaptic membrane receptors [Hockerman et al., 1997].

Pregabalin is a structural derivative of γ -aminobutyric acid (GABA). It acts mainly through presynaptic binding to the $\alpha 2\delta$ subunit of voltage-gated calcium channels that culminate in reduction of neurotransmitters release, e.g.,

glutamate, substance P, and calcitonin gene-related peptide (Taylor et al., 2007). Pregabalin relieve painful diabetic neuropathy (Rosenstock et al., 2004 and Freynhagen et al., 2005) and post-herpetic neuralgia (Dworkin et al., 2003 and Freynhagen et al., 2005). The blockade of calcium ion channels was proposed as a possible mechanism of action of Pregabalin [Taylor et al., 2007].

Gabapentin alter GABA uptake, or interfere with GABA transaminase. It appears to bind to an amino acid carrier protein and appears to act at a unique receptor. Gabapentin inhibits high-voltage activated calcium channels (Ferraro TN, Buono, 2005 and Taylor and Gee, 1998).

The movement of calcium into neurons may be the common denominator for the triggering and propagation of seizure activity. There is large body of evidence indicating that both high- and low-voltage-activated calcium channels and their subunits are involved in idiopathic generalized epilepsies (Zamponi et al 2010).

In this current study co administration of pregabalin, gabapentin and nifedipine may show synergistic effects on control of epilepsy and pain and open the gate for amelioration of these medical disorders that need lifelong treat-

*Corresponding author:

Ahmed Shata

Department of Clinical Pharmacology, Mansoura University, Mansoura, Egypt

(fax +20 (50) 2223613-124

E-mail: ahmedmhes@mans.edu.eg

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ment.

Materials and methods

All animal procedures and the experimental protocols were approved by the research and ethics committee of Mansoura University. Sixty male Swiss albino mice (weighing 20-25g) were obtained from Mansoura faculty of pharmacy. After one week of acclimatization to laboratory conditions, the animals were randomly assigned to experimental groups, 10 mice each. It housed under controlled conditioning ($25 \pm 1^\circ\text{C}$ temperature, 55-65% relative humidity, 12 h dark/light cycles). Food and water were allowed ad libitum during the study period. All tests were performed between 9:00 a.m. and 3:00 p.m. to minimize circadian influences on seizure susceptibility.

Chemicals and drugs

The following drugs were used in the current study: nifedipine (adalat[®]). Pregabalin, gabapentin, PTZ and all other chemicals were obtained from Sigma (St. Louis, MO, USA). Animals were divided into 6 main groups, 10 mice each. Positive control group, Sub-convulsive dose of PTZ (35 mg/kg, ip) was administered every other day, for a total of 11 injections [Inan et al., 2008]. Seizures induced were divided into behavioral categories (grade 0-6): grade 0, no behavioral seizures; grade 1, head nodding, head twitching; grade 2, myoclonic jerks; grade 3, head twisting, forelimb clonic convulsions; grade 4, kangaroo position; grade 5, falling down; grade 6, tonic convulsions according to the scoring system of Fischer and Kittner [Fischer et al., 1998]; Nifedipine treated group (suspended in a 1% aqueous solution of Tween 80 and implemented intraperitoneally (20 mg/kg) [Łuszczki et al., 2007]; Pregabalin treated group (40 mg/kg i.p.) [Akula et al., 2009]; Gabapentin treated group (200 mg/kg i.p.), [Akula et al., 2009]; Nifedipine + pregabalin group ([Łuszczki et al., 2007, Akula et al., 2009); and lastly Nifedipine+ gabapentin group (Łuszczki et al., 2007 and Akula et al., 2009)

The mean seizure stages were calculated for all groups after each PTZ injection.

Analgesimeter

Pain tolerance measurement right paw pad pressure tolerance (Analgesimeter: Ugo Basile, Italy). For assessment of the analgesic activity of the used drugs, pressure was applied by the analgesimeter on the mice pad of the right paw. The pressure was increased gradually (a certain number of grams per second until the mice either squeaks or tries to withdraw its limb). The force of pressure was continuously monitored by a pointer moving along a linear scale. Increased pressure tolerance of drug treated rats indicates analgesic activity of the administered drug [L.O. Randall et al., 1957 and Ward and Jones 1962].

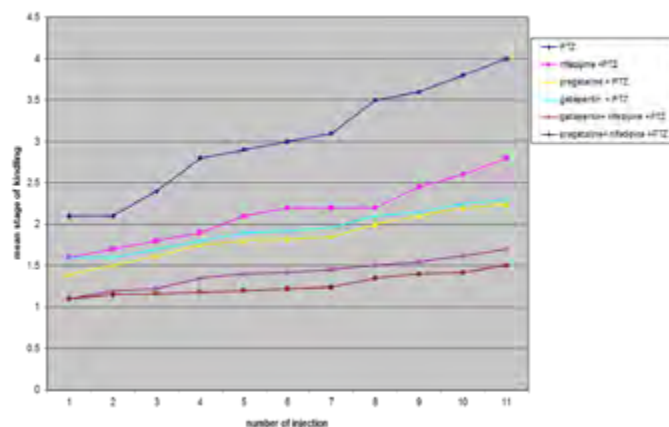
Statistical analysis

All data were expressed as the mean \pm SEM. Statistical significance was tested by one way analysis of variance (ANOVA) followed by Bonferroni post-hoc analysis. The confidence limit of $p < 0.05$ was considered statistically significant.

RESULTS

Kindling stage:

Fig 1: effect of tested drugs on kindling stage

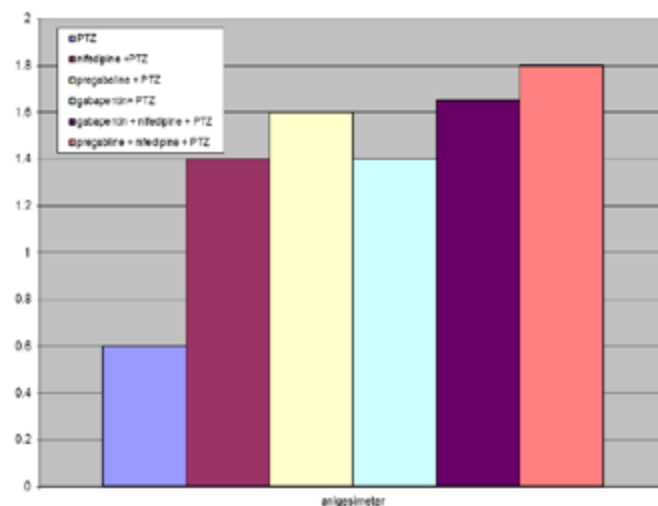


Gabapentin, pregabalin and nifedipine significantly decrease seizure activity in comparison with PTZ control mice ($p < 0.05$). On other hand, it show more significant decrease when it used together that confirm synergistic effect of nifedipine on pregabalin ($p < 0.009$), also it seen with gabapentin ($p < 0.011$).

Analgesimeter gm^{-1}

Gabapentin, pregabalin and nifedipine increase significantly pain tolerance in relation PTZ control mice ($p < 0.05$) and coapplication of both drugs either gabapentin with nifedipine or pregabalin with nifedipine show more synergistic significant effects on pain tolerance ($p < 0.001$)

Fig 2: effect of tested drugs on analgesimeter



DISCUSSION

Nowadays, the development of effective antiepileptic with fewer side effects and appropriate combination therapy is one of the most important objectives of drug researches. Therefore, new drug classes and combination therapy have been proposed.

Several animal models are used for seizures study but kindled seizures are widely accepted as an animal model of temporal lobe epilepsy (Szyndler et al., 2006) because it can study chronic nature of the induced seizure; a basic

feature of human epilepsy that is not found in other forms of induced epilepsy (Ali et al., 2005).

Many conditions was constantly associated with epilepsy including neurological pain that must raise our attention to ameliorate it beside control of epilepsy (Ottman et al., 2011). So, the relief of the associated epileptic pain is considered as crucial factor for proving the efficacy of newly antiepileptic drugs. Interestingly, the mechanism of seizure and its associated neurological pain is based on a final common path; the neuronal hyperexcitability. Most of antiepileptic drugs can reduce neuronal hyperexcitability through a shared mechanism of blocking ion channels (Zarremba et al., 2006).

The present study revealed four main findings: first, Gabapentin, pregabalin and nifedipine significantly decrease seizure activity in comparison with PTZ control mice. Secondly, it show a significant decrease when it used together that confirm synergistic effect of nifedipine on pregabalin. Thirdly, Gabapentin, pregabalin and nifedipine increase significantly pain tolerance in relation PTZ control mice and coapplication of both drugs either gabapentin with nifedipine or pregabalin with nifedipine show synergistic significant effects on pain tolerance.

Regarding pregabalin, it is was established as a newer anticonvulsant therapy (Taylor et al., 2007); it acts mainly on $\alpha 2\delta$ subunit of voltage-gated calcium channels at the nerve terminals preventing the releases of excitatory transmitters that precipitate the epileptic fits. In this work, beside its anticonvulsant effect, pregabalin has shown to significantly increase pain tolerance. Several previous studies have reported that pregabalin was proved to relief many subtypes of neurotic pain (Rosenstock et al., 2004, Freynhagen et al., 2005, and Dworkin et al., 2003).

An expected mechanism for the neurological pain relief is based on the closure of voltage gated calcium channels; the main mechanism of pregabalin action. It has been reported that the hyperexcitability present in epileptic patients play important role in neuropathic pain syndromes and epilepsy (Mona & Yasser 2012). It has to be noted that the key mechanism of action is mediated through Ca blocking agent rather than GABAergic systems (Ben-Menachem 2004).

On other hand, Gabapentin effectively reduced seizure activity in comparison with PTZ control mice. This results go through with previous reports that mentioned anticonvulsant role of gabapentin in clinical use since 1996 (Stefan & Feuerstein 2007). Gabapentin was reported to work through multiple mechanisms of action with inhibition of the high-voltage activated calcium channels being the most important reported one (Ferraro & Buono, 2005). This mechanism in turn could explain its action in increasing pain tolerance as previously reported in our work.

Lastly administration of calcium channels blocker; Nifedipine effectively reduced seizure activity in comparison with PTZ control mice. This effect has been reported in previous studies in different animal models of seizures (De Sarro et al., 1988 and Hunter et al., 1988). Also, blockade of calcium channels was proved effective against electrical-

ly-induced convulsions, amygdala-kindled seizures, quinolinic acid-induced seizure activity and hyperbaric oxygen-induced convulsions (Desmedt et al., 1976; Dolin et al., 1988; Vezzani et al., 1988). In the current study, nifedipine effectively increased pain tolerance ensuring the role of Ca channels blocker in controlling the neuropathic pain. In conclusion, the addition of calcium channel blockers; Nifedipine to newly anticonvulsant therapy (Pregabalin and Gabapentin) seems to be beneficial in controlling the attack of seizures and reducing the associated neuropathic pain that may enhance response, decrease side effects and shorten duration of therapy. Further researches are needed to evaluate this role in human studies.

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