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Original Article

Influence of β- blockers on the activity of some anti-epileptic drugs on convulsions induced by picrotoxin in mice

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Abstract

Adrenergic β-receptor blockers are widely used in clinic for the management of cardiovascular disease and some other illnesses. However, this group of drugs known to cause central nervous system side effects such as drowsiness, sleep disturbance, hallucination, migraine and tremors.As anti-epileptic drugs exert their action mainly through the inhibition of the central nervous system to decrease the firing and the excitability of neurons. Accordingly, β-blockers might influence the pharmacological activity of anti-epileptic drugs.
Aim:The aims of this study is to investigate the influence of β –blockerson the anti-convulsant activity of two anti-epileptic drugs, i.e. phenytoin and phenobarbital.
Methods: Three beta blockers with different β-receptor blocking selectivity and degree of solubility (atenolol, metoprolol, and propranolol) were injected intraperitoneally(IP) into mice either alone or in combination with phenytoin or phenobarbital. After 30 min mice were injected with picrotoxin (8mg/kg) to induce convulsions. Convulsion parameters recorded were; the onset of jerks, number of tonic and clonic convulsions, and % mortality.
Results: Picrotoxin produced 100% death in all control animals. However, mosttheanimals treated with antiepileptics alone or in combination with β-blockers were protected from death. The effect of phenytoin on the onset of convulsions was significantlyenhanced when it combined with β-blockers. However, in regard to phenobarbital only the increase was noticed with propranolol. Giving phenytoin with β-blockers improves its effect in reducing clonic convulsion, whereas, no change in phenobarbital activity when administered together with β-blockers. Combination of either phenytoin or phenobarbital with β-blockers did not result in any significant change in their ability to reduce tonic convulsions except when phenytoin co-administered with metoprolol a significant decrease was observed.
Conclusion: The administration of β-blockers in concomitant with phenytoin and phenobarbital increased their anticonvulsant activity. However, β-blockers alone could have some protective effect against convulsions.

Keywords: β-blockers, picrotoxin, convulsion, phenytoin, phenobarbital.

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Introduction

Adrenergic β-receptor blockers are a group of drugs works though antagonizing of β-adrenoceptors. They vary in their selectivity and the affinity to different types of receptors i.e. β1, β2, and β3(Farzam and Jan 2022a). Therefore, they could be selective to a particular type or even non selective such as; propranolol thehistorical non-selective β-adrenergic receptor antagonist. Beta adenoceptor blockers are one of the most commonly prescribed group of medications(Sellers and Lewis 2022), They are widely and effectively used for the management of cardiovascular disease such as; heart failure, hypertension, arrhythmias, and ischemic heart disease(Martínez-Milla et al. 2019). In addition they are frequently prescribed for some other disorders for example; anxiety and migraine (Laviolette et al. 2022). However, this group of drugs known to cause central nervous system (CNS) side effects such as; fatigue, depression, sleep disorders and nightmares, visual hallucinations, delirium or psychosis and Parkinson's disease like symptoms(Shah et al. 2020)(Ahmed et al. 2017, 2018).

The lipophilicity of a β-blocking agent is the main determinant to its distribution to the CNS.Highly lipophilic β-blockers such as propranolol and metoprolol, cross rapidly the blood brain barrier, in comparison to agents with low lipophilic properties such as atenolol (Shah et al. 2020). Therefore the lipo-solubility of aβ-blocker is linked to its side effects and the therapeutic efficacy on the CNS, as many reports showed beneficial outcome for β-blocker in treating some neuronal and psychiatric disorders(Liu et al. 2015).Nevertheless, lipid solubility is not the only factor determine the effect in CNS, other factors such as; the intrinsic activity of the agent to the receptor may control the extent of the effect(Magder et al. 1987).Most of β-blockers are metabolized majorly by CYP2D6, such as propranolol and metoprolol. Therefore the bioavailability of drugs that metabolized by the same enzyme could be altered and as a consequence their pharmacological effect (Maideen et al. 2021).Anti-epileptic drugs exert their action mainly through the inhibition of the central nervous system to decrease the firing and the excitability of neurons. Furthermore, the trend of actions of β-blockers on neurons is inhibitory (Farzam and Jan 2022b). Various mechanisms have been suggested to explain the inhibitory effects of these agents. For example,decreasing the release of glutamate in some brain areas by propranolol.(Okuda et al. 1999).

As β-blockers have different degree of selectivity and affinity to different typeof β-receptors and alsohave avaried degree of lipid solubility which determine their distribution to CNS. Therefore these could influence the side effects and the interactions of β-blockers with other CNS drugs. Hence, this research is to investigate a potential pharmacological interaction between these two important groups of drugs i.e. antiepileptics and β-blockers.

Materials and Methods

1-Animals

Male albinomice weighting (25–30g) were obtained from the Libyan Medical Research Center. Mice were kept in a room with an alternate 12-h dark/light cycle, under stable conditions of humidity (60 ± 5%) and temperature (25 ± 2 °C), with free access to regular diet chow and water. All animal experiments were approved by the ethical committee of thedepartment of pharmacology and clinical pharmacy.

2-Drugs

Propranolol was sourced from HikmaFarmaceutica Portugal, metoprolol and atenolol were obtained from Cipla India, phenytoin and phenobarbital injections were sourced from Abbot USA and,picrotoxin was obtained from Sigma Aldrich

3-Study design and treatments

Mice were randomly divided into 12 groups each group contains 6 mice (n=6). Mice in group one were injected with normal salineand works as control. Group 2 and 3 received either phenytoin or phenobarbital.Groups 4, 5 and 6; each received one of the β-blockers (atenolol or metoprolol or propranolol), whereasgroups 7, 8 and 9 each group were injectedwith a combination of phenobarbitaland one of 3 β-blockers. While groups 10, 11 and 12 each of them were administered a combination of phenytoin and one of the 3 β-blockers.

All the treatments were given by intraperitoneal (IP) administration in a volume of injection of 10 ml/kg. Phenobarbital and phenytoin were given in a dose of 25mg/kg while β-blockers were treated in a dose of 10mg/kg.

Picrotoxinin a dose of 8 mg/kg (Kumar Akula et al., 2007)was injected 30 minutes after the different treatments or saline. After injection each mouse was immediately placed individually in a transparent chamber and observed for the expression of convulsions for 30 minutes after picrotoxin injection. During this period, the parameters scored were onset of action clonic jerks, tonic extension, and death (Avallone et al., 2000; Mackenzie et al., 2002), (Zolkowska et al. 2012).

4-Statistical analysis

Descriptive statistical analysis was carried out for the generated data from different tests using SPSS (Software package, version 25). Also the data were tested to find out whether the observed samples are normally distributed using Kolmogorov-Samirnov-maximum deviation test for goodness of fit. If the parameters were normally distributed, the statistical analysis of results was done by usingt-test: paired two sample for means otherwise, groups where compared by using Mann-*Whitney*test. All experimental results obtained were represented as mean ± standard deviation of mean (S.D.Mean) of responses. For all tests, the values were considered as significant at a level of $p \leq 0.05$.

Results

As can be seen in (figure.1)set A (animals treated with β-blockers alone) were achieved significant protection against picrotoxin induced death;Metoprolol and propranolol reduced the percentage of mortality to 33% while atenolol produced a better effect as it lowered the mortality to only 17%. However, in set B; pretreatment of animals in with phenytoin alone or in combination with any of the three β-blockers (atenolol, propranolol or metoprolol) resulted in a 100% protection against picrotoxin induced mortality

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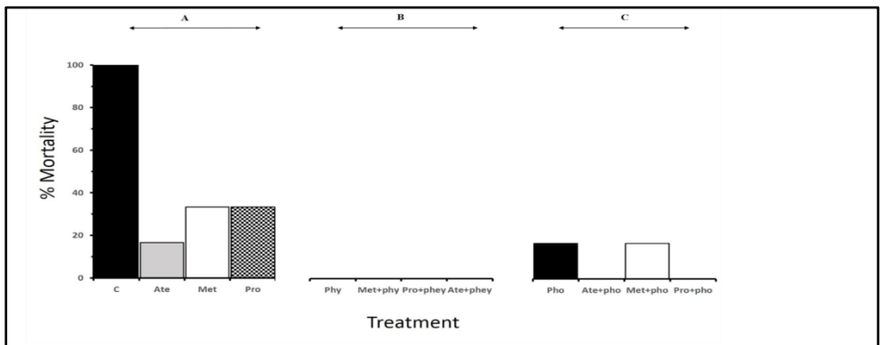

Figure.1. The percentage of mortality induced by picrotoxin during 30 min after pre-treatment with one of the following; normal saline (vehicle control) a β-blocker (atenolol, propropanol or metoprolo), phenytoin, phenobarbital or a β- blocker in combination with either phenytoin or phenobarbital. Data were represented as % mortality for (n=6). **A**; treated with β-blockers alone, **B**; phenytoin and β-blockers, **C**; phenobarbital and β-blockers. Control (**C**), atenolol (**Ate**), metoprolol (**Met**), propropanol (**Pro)**, phynetoin (**Phy**), phenobarbital (**Pho**).

In Set C; treating animals with phenobarbital reduced the mortality to 17%. Yet, combination of atenolol and propranolol withphenobarbital resulted in 100% protection against death. However, no change in protective ability of phenobarbital when it was combined with atenolol. The effect of various treatment on the onset of starting picrotoxininduced convulsions is demonstrated in (figure.2).

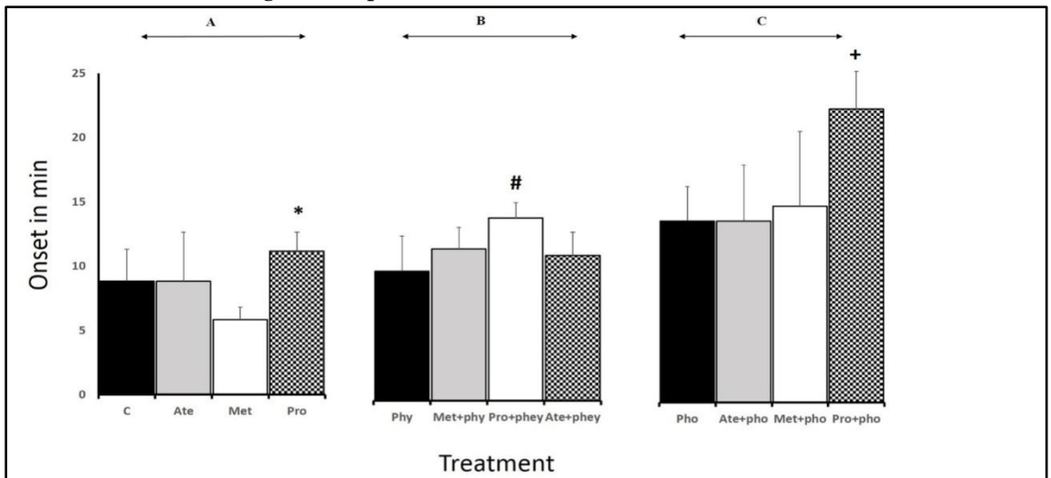

Figure.2. The onset of appearance of signs of convulsion after picrotoxin injection, animals were pre-treated with one of the following ; normal saline (vehicle control), a β-blocker (atenolol, propropanol or metoprolo), phenytoin, phenobarbital or a β- blocker in combination with either phenytoin or phenobarbital. Data were represented as means±SD of time in min for (n=6). * statically significant from control . # statically significant from phenytoin. + statically significant from pheobarbital**A**; treated with β-blockers alone, **B**; phenytoin and β-blockers, **C**; phenobarbital and β-blockers. Control (**C**), atenolol (**Ate**), metoprolol (**Met**), propropanol (**Pro)**, phynetoin (**Phy**), phenobarbital (**Pho**).

Data represented in set A,indicate that propranolol resulted in a significant delay in the time of convulsion occurrence. Although, atenolol and metoprolol did not influence the onset time.Set B at which animals were treated with phenytoin did not result in any change in onset, but when it was given with propranolol a significant increase in the onset of convulsion was noticed. However, no change was recorded after giving phenytoin together with either atenolol or metoprolol. Results in set C indicate that phenobarbital did not achieve any delay in the onset, but when it is given together with propranolol the onset time was significantly increased.Remarkably, the three tested β-blockers in set A were significantly reduced the tonic convulsions induced by picrotoxin(figure.3). Although, phenytoin (set B) and phenobarbital (set C) as expected decreased the number of tonic convulsions, on the contrary, giving either of them in combination with a β-blocker did not influence their activity on tonic convulsions. Exceptionally, metoprolol when given with phenytoin (set B) significantly increase the activity of phenytoin in reducing tonic convulsion.

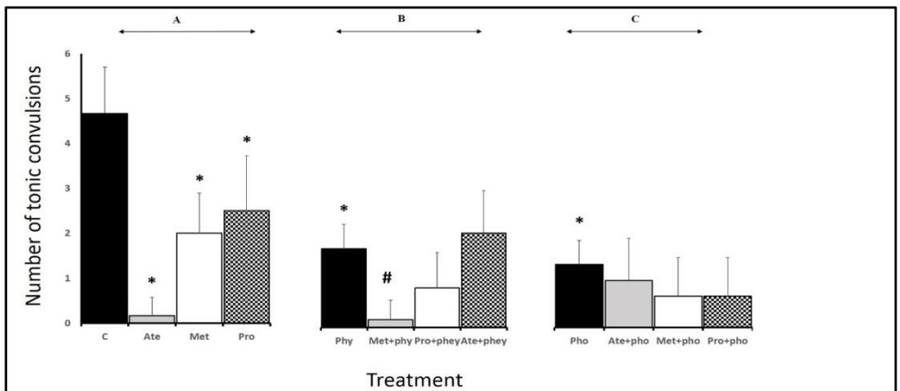

Figure.3. The number of convulsion after picrotoxin injection, animals were pretreated with one of the following ; normal saline (vehicle control), a β-blocker (atenolol, propropanol or metoprolo), phenytoin, phenobarbital or a β- blocker in combination with either phenytoin or phenobarbital. Data were represented as means±SD of number of tonic convulsion i for (n=6). * statically significant (*p≤0.05*) from control . # statically significant from phenytoin. **A**; treated with β-blockers alone, **B**; phenytoin and β-blockers, **C**; phenobarbital and β-blockers. Control (**C**), atenolol (**Ate**), metoprolol (**Met**), propropanol (**Pro**), phynetoin (**Phy**), phenobarbital (**Pho**).

Clonic convulsions data was also collected(figure. 4), unexpectedly, phenytoin (set B) raised the number of clonic convulsions, whereas, in combination with β-blockers the number was decreased. On the contrary, administrationof β-blockers with phenobarbital did not result in any change in the number of tonic convulsion (set C).

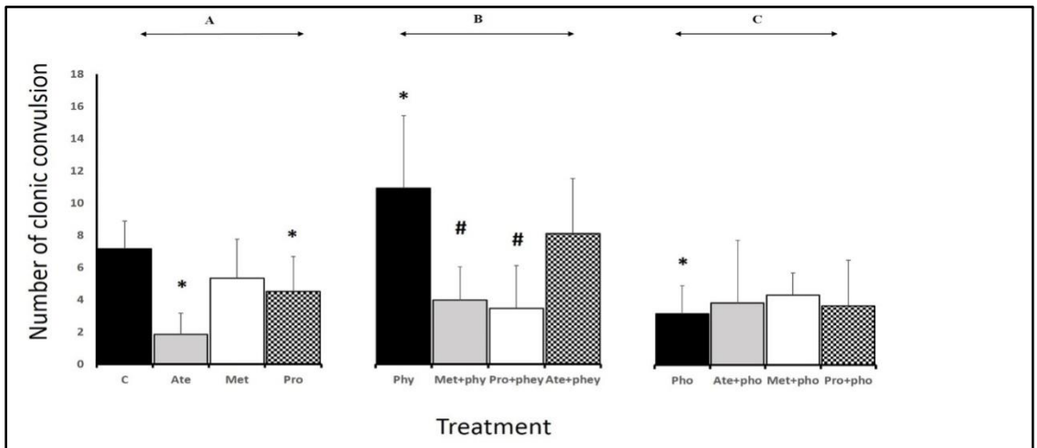

Figure.4. The number of clonic convulsion after picrotoxin injection, animals were pretreated with one of the following ; normal saline (vehicle control), a β-blocker (atenolol, propropanol or metoprolo), phenytoin, phenobarbital or a β- blocker in combination with either phenytoin or phenobarbital. Data were represented as means±SD of number of tonic convulsion i for (n=6). * statically significant (*p≤0.05*) from control . # statically significant from phenytoin. **A**; treated with β-blockers alone, **B**; phenytoin and β-blockers, **C**; phenobarbital and β-blockers. Control (**C**), atenolol (**Ate**), metoprolol (**Met**), propropanol (**Pro**), phynetoin (**Phy**), phenobarbital (**Pho**).

Remarkably, atenolol and propranolol (set A) were show a significant decrease in the number of convulsions.

Discussions

It is widely accepted epileptic seizure pathophysiologydependson the persistent increase of neuronal excitability. Therefore, it has been coined that research into the pathophysiology and therapy of epilepsy is mainly an examination of the balances between excitation and inhibition(Aminoff, Boller, and Swaab 2022). Furthermore, the current treatment approaches for the treatment of epilepsy mainly depend on enhancing the inhibition of brain excitatory signaling, i.e. enhancing GABA transmission or by blocking Na+ and/or Ca+2 channels (Ghosh et al. 2021). Beta adenoceptor blockers has been shown to exert some inhibitory action in brain through various mechanisms such as blocking Na+

channels (Chahine 2011), blocking Ca+2 channels (Tzingounis, Von Zastrow, and Yudowski 2010)and direct effect on central β-adrenergic receptors (Tzingounis, Von Zastrow, and Yudowski 2010).Therefore, many reports have suggested using of β-blocker to control some brain diseases (Liu et al. 2015). However, β-adrenergic blocking drugs are a widely used, well tolerated and effective treatment for a variety of cardiovascular and non-cardiovascular conditions. The most common side effects of β-blockers on brain in are fatigue and sleep disturbances, delirium, psychosis, and visual hallucinations (Goldner 2012). The severity of these side effects arein general directly related to their degree of lipophilicity(Cojocariu et al. 2021) and also their β-blocking selectivity (Kostis and Rosen 1987). Therefore, highly lipid soluble agents such as propranolol and metoprolol distribute radially through the blood brain barriers and reach brain, while, drugs with low lipo-solubility may not cross blood brain barriers. In the current study, all the tested β-blockers showed some activity against the symptoms of convulsion. For propranolol and metoprolol the effect may be anticipated as both are highly lipophilic and readily reach brain and they act at brain in a way similar to antiepileptic drugs (Ghosh et al. 2021). On the other hand,atenolol anticonvulsant activity may be explained; as a result of the action on β-receptor in periphery, or centrally without crossing the CNS bynitric oxide and hydrogen peroxide release (Laurens et al. 2019). All the three tested β-blockervariably potentiated theanticonvulsant activity of either phenobarbital that work by enhancing GABA transmission or phenytoin that blocks brain Na+ channels. However, propranolol seemed to have highest potentiation effect, followed by metoprololand atenolol comes last. Propranolol has the advantage of blocking β-receptor in non-selective fashion in addition to its action as membrane stabilizer and enhancer of brain inhibitory transmitters (Ghosh et al. 2021). Hence, the co-administration of antiepileptic drugs and β-blockers could improve the potency of epilepsy drugs, this is important,as β-blockers are extensively used in the management of cardiovascular diseases such ascardiac arrhythmiathat is frequently in comorbidity with epilepsy(Zaccara and Lattanzi 2019).

Conclusion

The administration of β-blockers (atenolol, metoprolol, and propranolol) in concomitant with phenytoin and phenobarbital may increase their anticonvulsant activity. However, β-blockers alone could have some protective effect against convulsions.

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