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Detection of the role of voltage-gated sodium channels in the mechanism of anticonvulsant action of Gabapentin in chicks (in-vivo)

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Abstract

Background:Because of the fact that the mechanism of anticonvulsant action of Gabapentin is not yet clear, so the aim of our present study was to determine whether the voltage-gated sodium channels (VGSC) may be correlate with its mechanism. To achieve this goal, Cypermethrin was chosen as a (convulsion inducer) resulting from its prolongs the opening of (VGSC) in order to interfere with Gabapentin .

Method: The experiment animals were divided into four groups. The first group was treated with a single dose of Cypermethrin (1000 mg / kg, orally), while the second and third group were treated with a single dose of Gabapentin (100 mg / kg, orally)15 or 30 minutes before the Cypermethrin treatment respectively. The fourth group was treated with Gabapentin alone in a dose of (100 mg / kg, orally) .After the end of treatment of the chicks were transferred to the cages to be monitored individually and recorded percentages of appearance of nervous signs and the percentage of each mortality and protection against mortality.

Results: Chicks treated with Cypermethrin alone (1000 mg / kg, orally)showed nervous signs which included the jerking movements of the leg and wings (clonic convulsion) and whole body tremors accompanied with opisthotonos at percentages (80%, 100%,60%) respectively that end with death at (100%) , but the pretreatment of chicks with Gabapentin (100 mg / kg, orally) resulted in time-dependent protection against Cypermethrin-induced nervous signs and mortality,which representing by significantly decrease in the percentage of each clonic convulsion,whole body tremors and mortality to (0%, 20% and 20%) respectively and accompanied by significant increase the percentage of protection of chicks against mortality to (80%) in the group that pretreatment with Gabapentin 30 minute before administration of Cypermethrin compared with control group (Cypermethrin alone).

We conclude from our results that recorded for the first time the Gabapentin provided protection to chicks against Cypermethrin-induced nervous signs and mortality , this result may be related to effect of Gabapentin on (VGSC). Thus, the currently study will open the way to use Cypermethrin as a model in scientific research that detect the mechanisms of action of anticonvulsant drugs .

Keywords: Gabapentin , Cypermethrin, Anticonvulsant, Voltage-gated sodium channels, Chicks

الكشف عن دور قنوات الصوديوم ذات الجهد الكهربائي في آلية عمل الكابابنتين المضاد للاختلاج العصبي في افراخ الدجاج (داخل الجسم الحي)

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الخلاصة

الخلفية:بالنظر لكون الية عمل عقار الكابابنتين كمضاد للاختلاجات العصبية غير واضحة لحد الان , لذلك كان الهدف من دراستنا الحالية هو الكشف عن امكانية ارتباط قنوات الصوديوم ذات الجهد الكهربائي بآلية عمل الكابانتين ، ولتحقيق ذلك الهدف تم اختيار السايبرمثرين (كمحدث للاختلاجات العصبية) والناتجة من اطالته لفتح قنوات الصوديوم لكي يتداخل مع الكابابنتين.

الطريقة: قسمت حيوانات التجربة الى اربعة مجاميع . عوملت المجموعة الاولى بجرعة منفردة من السايبرمثرين (1000 ملغم/كغم ,عن طريق الفم) في حين عوملت المجموعة الثانية والثالثة بجرعة منفردة من الكابابنتين (100 ملغم/كغم ,عن طريق الفم) قبل 15 او 30 دقيقة من المعاملة بالسايبرمثرين وعلى التوالي .وعوملت المجموعة الرابعة بالكابابنتين لوحده بجرعة (100 ملغم/كغم ,عن طريق الفم) . بعد الانتهاء من معاملة الافراخ تم نقلها الى الاقفاص وذلك لغرض مراقبة كل حيوان بصورة منفردة لتسجيل النسبة المئوية لظهور الاعراض العصبية والنسبة المئوية لكل من الهلاك والحماية من الهلاك .

النتائج: اظهرت الافراخ المعاملة بالسايبرمثرين لوحده (1000 ملغم / كغم ، عن طريق الفم) علامات عصبية شملت حركات اهتزازية في الساق والأجنحة (اختلاج عصبي من نوع الكلوني) وارتجاف كامل الجسم ومترافقة مع حدوث التواء الرقبة بنسب مئوية (80 ٪ ، 100 ٪ ، 60 ٪) على التوالي وتنتهي هذه العلامات بالهلاك وبنسبة (100 ٪) .في حين ادت معاملة الافراخ مسبقا بالكابابنتين (100 ملغم / كغم ، عن طريق الفم) الى حماية الافراخ وبطريقة معتمدة على الوقت ضد حدوث العلامات العصبية والهلاك المحدث بالسايبرمثرين ,تمثلت في حدوث انخفاض معنوي في النسبة المئوية لكل من الاختلاج العصبي الكلوني والرجفة والهلاك إلى (0 ٪ ، 20 ٪,20 ٪) على التوالي ومصحوبة بحدوث زيادة معنوية في النسبة المئوية لحماية الافراخ من الهلاك إلى (80٪) في المجموعة المعاملة مسبقا بالكابابنتين قبل 30 دقيقة من إعطاء السايبرمثرين بالمقارنة مع مجموعة السيطرة (سايبرمثرين وحده).

في الختام ،نستنتج من نتائج دراستنا الحالية والمسجلة لأول مرة نجاح الكابابنتين في توفير حماية للافراخ ضد العلامات العصبية والهلاك المحدث بالسايبرمثرين ، وقد تكون هذه النتيجة مرتبطة بتأثير الكابابنتين على قنوات الصوديوم ذات الجهد الكهربائي. وبذلك سوف تفتح الدراسة الحالية الطريق لاستخدام السايبرمثرين كنموذج في الابحاث العلمية الخاصة بكشف اليات عمل الادوية المضادة للاختلاجات العصبية .

كلمات مفتاحية: كابابنتين، السايبرمثرين ، مضاد للاختلاجات العصبية، قنوات الصوديوم ذات الجهد الكهربائي، ،افراخ دجاج.

Introduction

Gabapentin (Neurontin) is a synthetic analogue of the inhibitory neurotransmitter a gamma-amiobutyric acid (GABA) that would be sufficiently lipid soluble to penetrate the blood–brain barrier to exert its anticonvulsant effect that is also effective in the treatment of neuropathic pain (1,2)

However, its a structural analog of the(GABA).It does not interact with GABA receptors and is not a GABA agonist, and does not affect GABA uptake or degradation (3,4).Therefore,the mechanism of anticonvulsant action of Gabapentin has remained poorly understood (5).As its known the activity of the voltage-gated sodium channels (VGSC) has long been linked to disorders of neuronal excitability such as convulsion,and also because of the researchers obtained some promising results with Gabapentin to modulate (VGSC) expression by interfering with the trafficking pathway(in vitro) (6).Thus, based on what was mentioned above, our current study aimed to perform (in vivo) research on the possibility of correlating (VGSC) with the mechanism of anticonvulsant action of Gabapentin, to achieve this goal, Cypermethrin (type II a synthetic pyrethroid insecticides) (7,8)was chosen to interfere with Gabapentin,because the mechanism of Cypermethrin-induced convulsion is associated with the prolongation of the opening (VGSC) allowing more sodium ions to cross and

depolarize the neuronal membrane leading to hyperexcitation of the central nervous system and induce convulsion (9,10).

Materials and Methods

Day old Ross broiler chicks of both sexes were purchased from a certified local hatchery in Nineveh province and they were kept in the breeding cages of the animal house of the College of Veterinary Medicine/Mosul University to acclimatize to the laboratory conditions until the age of two weeks when the experiments were done. The chicks were housed in a room with provided the appropriate conditions like temperature (32-35 ° cm), ventilation and lighting with feed and water were provided ad libitum.Their weights ranged between 100-150 g and were observed to be close to weights in the experiment.

In this experiment,we used the chicks as a model (11) to detect the mechanism of anticonvulsants action of Gabapentin (Mylan-Alphapharm Ply-Limited- Aust-ralia) at dose (100 mg / kg, orally) was based on (12). Cypermethrin (10%-Star Vet –Jordan) was used as a convulsion inducer at dose (1000 mg / kg, orally) was based on (13) .Both Gabapentin and Cypermethrin were dissolved in distilled water, at a volume of 5 ml/kg. All doses of Gabapentin and Cypermethrin were freshly prepared before each experiment. All experiments complied with our institutional regulations addressing animal use, attention and humane care which are based on (14).

Twenty chicks were divided into four equal groups.The Cypermethrin and vehicle (distilled water) were administered a single dose to animals of control group (I).The Gabapentin was given a single dose 15 or 30 minute before administration of Cypermethrin respectiv-ely to animals of groups (II) and (III) . Gabapentin alone was given a single dose to animals in group (IV).

After the end of treatment of the chicks were transferred to the cages to be monitored individually and recorded percentages of appearance of nervous signs and the percentage of each mortality and protection against mortality .

Equation for calculating the percentage of protection against mortality

Total number of animals in the group – Number of dead animals in the group / Total number of animals in the group

Statistical analysis:

Nonparametric data were analyzed using the Fisher test .A probability level of $P<0.05$ was selected as a criterion for - statistically significant differences (15).

Results:

Cypermethrin(1000mg/kg,orally) was induced nervous signs in chicks: they consisted of an initial excited state which demonstrated by appearance of a symmetrical jerky movements of the legs and wings on the one side of the body in a consistent and frequent manner every (2-3) seconds (clonic convulsion) (80%), whole body tremors (100%) and opisthotonos (60%)accompanied with increase in percentage of mortality which reached to (100 %) as presented in table 1,2.

Pretreatment of chicks with Gabapentin (100mg/kg,orally) 30 minute before giving Cypermethrin(1000mg/kg,-,orally) elicited reversal of the convulsant actions of Cypermethrin which was recorded for the first time in our study, which demonstrated by significantly decrease in percentage of the appearance of clonic convulsion to (0%), whole body tremors to (20%) and mortality to (20 %**)** leading to the significant increase in the percentage of protection of chicks against mortality to (80%) compared to control group (Cypermethrin alone) as presented in table 1,2 **.**

In the current study,the protective effect of Gabapentin against Cypermethrin-

induced nervous signs in chicks was a time-dependent,which demonstrated by the decrease in percentage of occurance of clonic convulsion (jerky movements) to (0%) accompained with significantly decrease in percentage of appearance of whole body tremors to (20%) in the group pretreatment with Gabapentin 30 minute before administration Cypermethrin compared to group that pretreatment with Gabapentin 15 minute befor cypermethrin as presented in table 1 .

Chicks were treatment with Gabapentin (100 mg/kg,orally) alone in the group (IV) showed signs like sedation and feather plumped .

Table-1: Effect of Gabapentin on Cypermethrin-induced nervous signs in chicks

No.	Treatment groups	Nervous signs %		
		clonic convulsion	whole body tremors	opisthotonos
1	**Cypermethrin (1000 mg/kg,orally) (Control)**	80% (4/5)	100% (5/5)	60% (3/5)
2	**Pretreatment with Gabapentin (100 mg/kg,orally)15 minute before administration of Cypermethrin**	20% (1/5)	100% (5/5)	20% (1/5)
3	**Pretreatment with Gabapentin (100 mg/kg,orally) 30 minute before administration of Cypermethrin**	*0% (0/5)	*** a** 20% (1/5)	20% (1/5)

All the values are mean ± SE values of 5 animals in each groups .

* significantly different (P<0.05) compared with control group (Cypermethrin alone).

a significantly different (P<0.05) compared with group that pretreatment with Gabapentin 15 minute before administration of Cypermethrin .

Table- 2:The protective effect of Gabapentin against Cypermethrin –induced mortality

No.	Treatment groups	No. of dead animals in the group/ Total No. of animals in the group	Mortality %	Protection %
1-	Cypermethrin (1000 mg/kg,orally) (Control)	5/5	100 (5/5)	0
2-	Pretreatment with Gabapentin (100 mg/kg,orally) 15 minute before administration of Cypermethrin	2/5	40 (2/5)	60
3-	Pretreatment with Gabapentin (100 mg/kg,orally) 30 minute before administration of Cypermethrin	1/5	20* (1/5)	80*

All the values are mean ± SE values of 5 animals in each groups .

* significantly different ($P<0.05$) compared with control group (Cypermethrin alone).

Discussion

The mechanism of anticonvulsant action of Gabapentin has remained poorly understood (5) . For this reason, the purpose of our current research was to determine the possibility of the voltage-gated sodium channels (VGSC) may be correlated with the mechanism of anticonvulsant action of Gabapentin . To achieve this goal, Cypermethrin was chosen as a (convulsion inducer) resulting from its prolongs the opening of (VGSC) where these channels are considered to be the main targets for its action (9,10) in order to interfere with Gabapentin .

In the present study Cypermethrin(1000mg/kg,orally)produced nervous signs in chicks like clonic convulsion,whole body tremors and opisthotonos was similar to that reported in chicks that treated with Cypermethrin (16,17). This nervous signs that induced by Cypermethrin are mediated by its prolongs the opening of (VGSC), a major site of its action, allowing more sodium ions to cross and depolarize the neuronal membrane which results in continuous nerve stimulation (10) . It leads to tremors and lose of nervous control like convulsion (9) .

In this work, pretreatment of chicks with Gabapentin provide protection against Cypermethrin –induced nervous signs and mortality. As mentioned earlier in the paragraph above, the nervous signs like (convulsion) produced by Cypermethrin occur by prolonging the opening of (VGSC), thus the pathway of anticonvulsant action of the Gabapentin possibly during inhibition of (VGSC) leading to a discontinuation in the entery of more sodium ions to the neuronal membrane ,thus, preventing the occurance of nervous signs and mortality . This interpretation was supported by researches (6),where they suggested that, the Gabapentin modulation the (`VGSC) expression by interfering with the trafficking pathway.

With regard to the relationship of Gabapentin with channels other than sodium channels,the researches (18,19) pointed out that,the Gabapentin appears unable to impact on the voltage-dependent calcium channel trafficking or function in cultured hippocampal neurons (in vitro),this conclusion confirms our interpretation mentioned above about the association of the (`VGSC) with protective effect of Gabapentin against Cypermethrin –induced nervous signs and mortality in chicks (in vivo).

It is important to mention that,our results revealed the success of the chicks model in detecting the pharmacological effects (anticonvulsant effect) of Gabapentin which representing by completely abolished the clonic convulsion activity that reach to (0%) while, the percentage of the appearance other signs,such as whole body tremors and opisthotonos decrease to (20% and 20%) respectively in the group that pretreatment with Gabapentin 30 minute before administration Cypermethrin.

The outcome of our current study has been supported by the results of a case report of a patient who was poisoned by permethrin (20)which its belongs to the same group of Cypermethrin and also works on lengthening the opening (VGSC). The patient did not respond to atropine treatment.Subsequently,benzodi-azepine therapy was initiated. The patient remained comatose and on mechanical ventilation with poor deep tendon reflexes, muscle weakness, pinpoint pupils, increased secretions and diarrhea.Then,the patient was started on Gabapentin. After eight days, the patient was extubated after demonstrating improved responsiveness, normal pupils, and decreased secretions.

The anticonvulsant effect of Gabapentin in chicks was time dependent,this effect was best when its given 30 minute before treatment with the Cypermethrin .This result probably related to pharmacokinetics effects of Gabapentin, it is possible that the concentration of the drug in blood plasma reached its therapeutic concentration when given 30 minutes before the Cypermethrin, Consequently may be lead to the modulation of the function of (VGSC) (6) and prevent the incidence of clonic convulsions (jerky movement) accompained with increase the percentage of protection against mortality in chicks to (80%).

The signs like sedation and feather plumped were induced by Gabapentin (100 mg/kg,orally) alone in chicks agree with the previous experimental findings (21) in Hispaniolan Amazon parrots.

Conclusions

We conclude from the our results that recorded for the first time the Gabapentin provided protection against Cypermethrin-induced nervous signs and mortality in chicks,which may be related to its effect on the (VGSC). Thus, this study will open the way to use Cypermethrin as a model in scientific research that detect the mechanisms of action of anticonvulsant drugs .

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Conflict of interest

There exists no conflict of interest.

Authors contribution

Both authors have equal contribution for this manuscript.

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