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In Vitro Anticonvulsant Action of 2-Arachidonyl Glycerol

Abdulmonem A. Al-Hayani, (DipFMS, PhD)

Department of Anatomy, Faculty of Medicine
King Abdulaziz University, Jeddah, Saudi Arabia
hayani30@hotmail.com

Abstract. Previous studies had shown that the exogenous cannabinoids can induce anticonvulsion, which was believed to be mediated through the activation of central cannabinoid Type 1 receptors. Moreover, the endogenous cannabinoid anandamide has shown anticonvulsant properties in the *in vitro* preparations. The current study used 27 adult Sprague-Dawley rats to investigate the effects of another endogenously occurring cannabinoid called 2-arachidonyl glycerol on epileptiform activity induced by picrotoxin. Extracellular recordings were made from the pyramidal cell layer of the CA1 region of hippocampal slices maintained in an interface type recording chamber. Stimulation was performed by using single pulses. It evoked population spikes of approximately equal amplitude. Using single pulse stimulation, perfusion of 500 nM picrotoxin caused an increase in the amplitude of the first population spike, and caused epilepsy by introducing a second or multiple population spikes. In the presence of picrotoxin, 2-AG reduced the amplitude of the population spikes, thus reducing the epilepsy. The CB_1 receptor antagonist, AM281 (500 nM) had no effect on responses recorded in the presence of picrotoxin, but totally blocked the effect of subsequently perfused 2-AG. The current results showed that 2-AG caused anticonvulsion in the *in vitro* preparations. The results will open avenues for cannabinoid receptors as possible future therapeutic targets.

Keywords: 2-arachidonyl glycerol, Endogenous cannabinoids, Anti-convulsion, Hippocampal slice.

Correspondence & reprint requests to: Dr. Abdulmonem A. Al Hayani
P.O. Box 80205, Jeddah, 21589 Saudi Arabia
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Introduction

Although epilepsy is one of the most common neurological conditions, the cellular mechanisms accounting for the neurological abnormalities are not fully understood[1]. The epileptic focus has been shown to contain elevated glutamate levels, and although glutamate antagonists have been investigated as potential antiepileptic drugs, this has as yet proved unsuccessful[2]. In contrast, pharmacological enhancement of GABAergic transmission has proved a powerful mechanism of action for antiepileptic drugs[3]. Therefore, a clear understanding of the pathophysiology of seizure initiation and termination is necessary for our ability to manage seizure disorders and for the potential development of novel anti-epileptic agents. Previous research in our laboratory[4-5] and others[6-7] have shown that cannabinoid compounds such as WIN55, 212-2 and anandamide are anti-epileptic compounds in both *in vitro* and *in vivo* studies. We further demonstrated that the anti-epileptic effect of cannabinoids was mediated through the central cannabinoid Type 1 receptors (CB_1)[4].

To date, two separate cannabinoid receptors (CB_1 and CB_2) have been identified. CB_1 was cloned in 1990, whereas CB_2 was cloned in 1993[8]. CB_1 was found to be present in brain regions such as the hippocampus and is known to mediate the psychoactive effect of cannabinoids, whereas CB_2 receptors were found in the immune system[8-10]. Following the discovery of cannabinoid receptors, two endogenously occurring agents were discovered in 1992 and 1995[11], called arachidonylethanolamide (anandmide) and 2- arachidonylglycerol (2-AG), where there is evidence to suggest that both can serve as neurotransmitters[12]. As our previous studies had shown, an anti-epileptic effect of the cannabinoid CB_1 agonists WIN55, 212-2 and anandamide[4-5], the next logical step was to confirm this effect by testing another endogenous cannabinoids (2-AG) in the anti-epileptic scenario, which is the aim of the current study.

Methods

The methods used in the current study are a standard electrophysiological technique that has been used by many previous studies[4-5,13]. Twenty-seven young adult Sprague-Dawley rats aged from 4 to 6 weeks were used in the current study. After general anesthesia with halothane, they were decapitated and the brain was removed from the skull

and submerged in oxygenated, cold (under 5ºC) artificial cerebrospinal fluid (aCSF). The hippocampus was dissected out and chopped transversely on a McIIwain tissue chopper forming slices 400µm thick. The slices were placed onto moist filter paper in a petri dish and maintained in a well-oxygenated and humidified chamber. After at least one hour, slices were transferred to an interface-type recording chamber which was continuously perfused with aCSF at a rate of 2 ml/minute. The temperature was maintained between 28-30ºC. A pulled bipolar stimulating electrode was used to stimulate the Schaffer collateral commissural fibers, and evoked population spikes were recorded from the cell body layer of the CA1 region of the hippocampus using a glass capillary microelectrode filled with aCSF (Fig. 1). Half-maximal population spikes were then evoked at 30 second intervals until a stable baseline of at least 20 minutes was established. Data was stored and analysed using the LTP program[14].

2-AG and AM281 were obtained from Tocris (Bristol, UK). Stock solutions of 2-AG were made up in alcohol and stored at 4ºC. When required, they were diluted with aCSF to obtain the required concentration. AM281 (the cannabinoid CB_1 receptor antagonist) was made up as a 10 mM stock solution in dimethyl sulphoxide (DMSO) and diluted in aCSF as required.

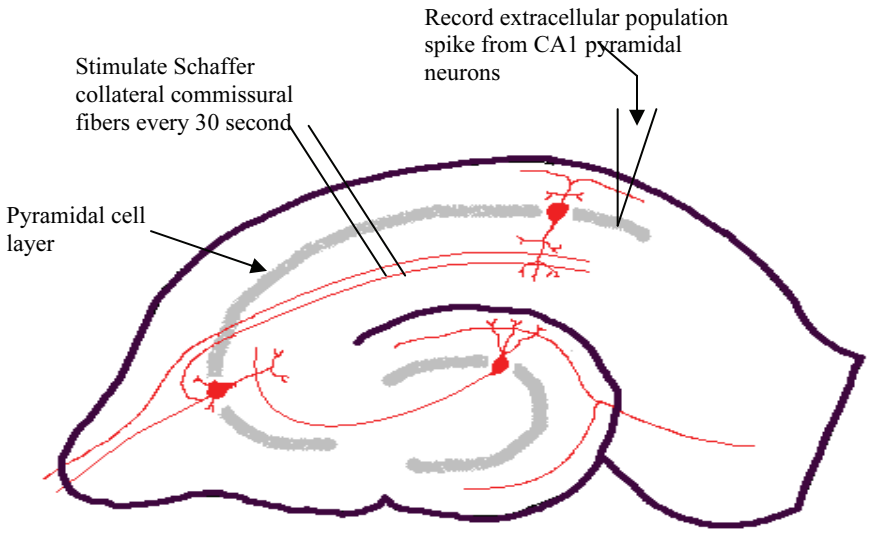

Fig. 1. 400µm thick hippocampal slice showing the positions of both stimulating electrode and the recording electrode from the pyramidal cell layer.

The convulsant poisonous plant derivative 'picrotoxin' was used to induce convulsion. Picrotoxin has been widely used to induce epilepsy in *in vitro* preparations[12]. The multiple population spikes recorded after the application of picrotoxin were named PS1, PS2, *etc* (Fig. 2). The paired "Student's'" *t*-test of the INSTAT program was used for statistical analysis. Each slice included in the results came from a different rat. A *P* value of less than 0.05 was considered statistically significant.

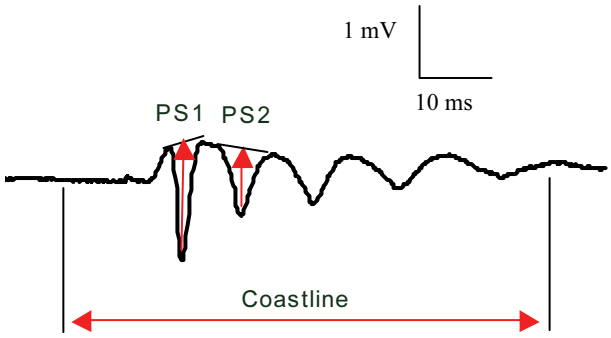

Fig. 2. An example of a synaptic response recorded from the CA1 region of the hippocampal slice showing multiple population spikes after application of picrotoxin (500nM). PS1 is the first population spike and the red upward arrow indicates the amplitude (height) of the population spike in millivolt (mV). PS2 is the second population spike and the red upward arrow indicates the amplitude (height) of the population spike in mV. Inset shows the time scale of the synaptic response.

Results

1. *Picrotoxin Induced Convulsions in the Rat Hippocampal Slice*

After recording a steady baseline for at least 20 min, perfusion of picrotoxin (500 nM) for 30 min caused epilepsy by introducing a second population spike (PS2) and increasing the amplitude of both PS1 and PS2. The amplitude of PS2 increased to 157 ± 7% of the control baseline (n=8, Fig. 3).

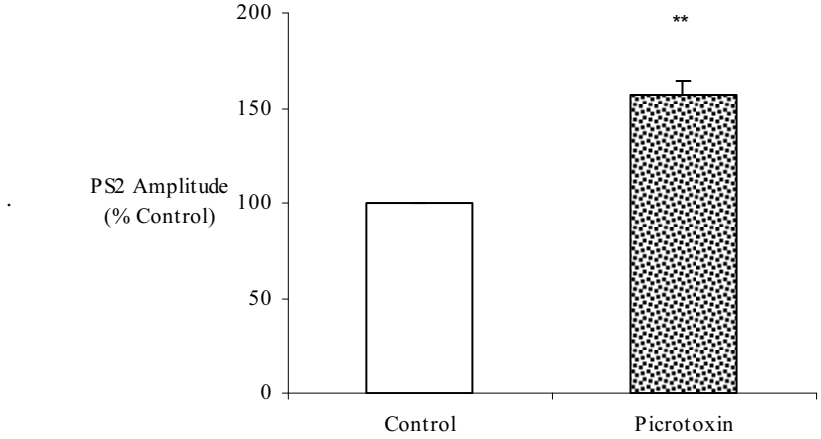

Fig. 3. Perfusion of picrotoxin (500nM), indicated by the dotted bar, for 30 min caused a significant increase ($p< 0.05$) of the amplitude of PS2 (n=8). Data are presented as mean amplitude.

2. *2-AG is Anticonvulsant in the in vitro Model of Epilepsy*

After a steady baseline has been recorded for at least 20 min, picrotoxin (500 nM) was perfused until a second population spike was introduced and had reached a steady baseline. The perfusion of picrotoxin increased the amplitudes of PS1 and PS2. 2-AG (10 μM) was then perfused for 30 min. Perfusion of 2-AG (10 μM) reduced the amplitude of PS1 to 56.4 ± 6% of the picrotoxin baseline. The amplitude of PS2 was reduced to 64 ± 9% of the picrotoxin baseline (n=10, Fig. 4). 2-AG therefore showed a strong anticonvulsant effect.

3. *2-AG's Anticonvulsant Effect is Mediated by Cannabinoid's CB_1 Receptor Activation*

A pretreatment dose of the cannabinoid CB_1 receptor antagonist, AM281 (500 nM) was used to test if the effect of 2-AG was mediated through the central cannabinoid CB_1 receptors or not. Stable control response was obtained prior to the perfusion of 500 nM picrotoxin. Once a stable second population spike was obtained, the CB_1 receptor antagonist AM281 (500 nM) was perfused for 30 min. This was followed by a perfusion of 2-AG (10 μM) for 30 min. AM281 alone did not affect the amplitude of the PS1 and PS2. After 30 min perfusion of AM281, the

amplitude of PS2 was 96 ± 6% of the picrotoxin baseline (*i.e.* no significant change). AM281 markedly reduced the effect of the subsequently perfused 2-AG (10 µM). The perfusion of 2-AG (10 µM) for 30 min resulted in a very small reduction of the amplitudes of PS1 and PS2 (92 ± 3% and 93 ± 8%, respectively) which were not statistically significant (n=7, $p<0.05$, Fig. 5).

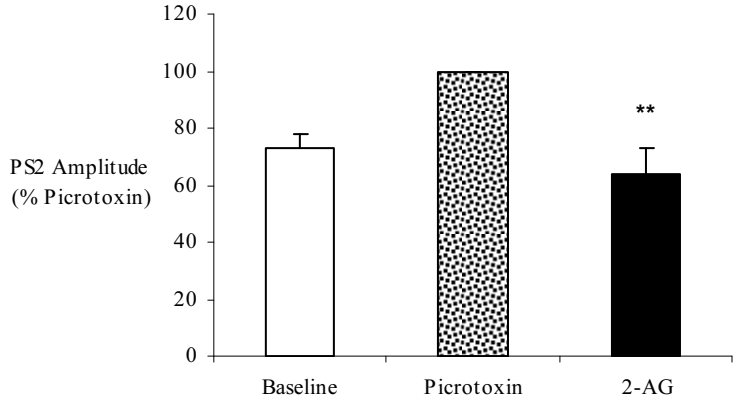

Fig. 4. Perfusion of the endogenous cannabinoid 2-AG (10 µM), indicated by the black bar, for 30 min has no significant effect ($p< 0.05$) on the amplitude of PS2 in slices where epileptiform activity had been induced by perfusion of picrotoxin (500nM), indicated by the dotted line, n=10. Data are presented as mean amplitude.

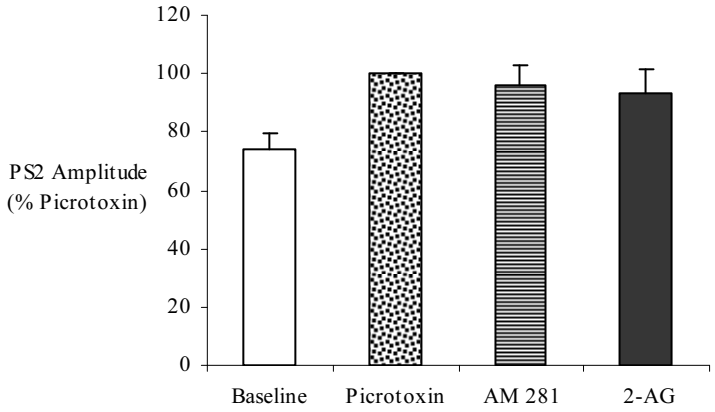

Fig. 5. The CB_1 receptor antagonist AM281 (500nM and striped bar) totally blocked the effect of subsequently perfused 2-AG (10 µM and black bar), in slices where epileptiform activity had been induced by perfusion of picrotoxin (500nM and dotted bar). Data are presented as mean amplitude ($p< 0.05$, n=7).

Discussion

The current study investigated the effects of 2-AG on epileptiform activities induced by picrotoxin. 2-AG showed a potent anticonvulsant action that has been mediated by the activation of the cannabinoid CB_1 receptor.

2-AG is the second putative endogenous ligand for cannabinoid receptors to be identified[15]. It belongs to a class of fatty-acid derivatives of N-arachidonyl-phosphatidylethanolamine[8,15]. As many previous studies have shown that elevated intracellular calcium accompanies seizure activity[16-17], the depolarization and calcium dependent synthesis of endogenous cannabinoids including 2-AG, therefore, suggests that this system could play a significant role in controlling seizure activity[16-17]. One of the major brain regions involved in epileptogenesis and seizure disorders is the hippocampus[18], which justify the selection of the hippocampus in the current study.

The most likely cannabinoid receptor involved in the 2-AG anticonvulsant effect is CB_1 receptor[19]. The cannabinoid CB_2 receptor is unlikely to be involved since this receptor is not present in the brain[8,19]. Moreover, the current study revealed that CB_1 receptor antagonist (AM 281) is selective for the cannabinoid CB_1 receptor, with negligible binding at cannabinoid CB_2[8]. Thus, the current data strongly suggests the cannabinoid CB1 receptor as the mechanistic site of action controlling the anticonvulsant effects of endocannabinoids. This is in support of previous research using other endocannabinoids to induce anti-convulsion[5,20-21].

In conclusion, the current study is in support of the previous evidence that endocannabinoids play a direct physiological role in modulating seizure activities. In addition, these data further establish the cannabinoid CB_1 receptor and the endogenous cannabinoid system as a potential treatment target for the control of epilepsy.

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مادة ٢–أ ج تقلل من التشنجات الصرعية في التجارب على الشرائح المخية

عبد المنعم عبد السلام الحياني

قسم التشريح ، كلية الطب ، جامعة الملك عبدالعزيز

جـــــدة – المملكة العربية السعودية

المستخلص. لقد أثبتت الدراسات السابقة أن مستخلصات الكانابينويدز المصنعة قادرة على تقليل التشنجات الصرعية، وذلك عن طريق تحفيز مستقبلات (س ب ١) العصبية. كما أثبتت الدراسات أن مادة الأناندمايدز المفرزة داخل الجسم لها مقدرة أيضا في تقليل التشنجات الصرعية. ولتأكيد ذلك قامت الدراسة الحالية بدراسة متستخلص آخر من مستخلصات الكانابينويدزالمفرزة داخل الجسم يسمى ٢–أ ج، واستخدمت لذلك ٢٧ فأرا لدراسة تأثير هذه المادة على التشنجات الصرعية.

ولاختبار تأثير هذه المادة، تم استئصال قرن آمون (الهيبوكامبس) من مخ ذكور الفئران البيضاء، وتقطيعه إلى شرائح بسمك ٤٠٠ مايكرون، وذلك لدراستها بالوسائل الكهروفسيولوجية، حيث تم تسجيل الاستجابة العصبية قبل وبعد إضافة الأدوية المختلفة، كما تم قياس طول الاستجابة العصبية، والتغيير فيه قبل وبعد إضافة الأدوية المختلفة. و تم تحليل النتائج باستخدام برنامج "إل تي بي".

بعد إحداث تشنجات صرعية نتيجة إضافة مادة البكروتوكسين إلى الشرائح المخية، تمّ إضافة أراكيدونيل جليسرول (٢–أج)، والتي قامت بتقليل التشنجات الصرعية بشكل مهم إحصائيا. وعند

إضافة مادة "ايه ام ٢٨١" لم تتمكن مادة ٢-أج من منـــع حـــدوث التشنجات.

وأثبتت الدراسة قدرة مـــادة ٢-أج علـــى تقليـــل التـــشنجات الصرعية بشكل مهم وملحوظ، مما يدلل على الدور الكبير الذي قد تلعبه مستقبلات (س ب ١) العصبية في علاج الأمراض العـــصبية مستقبلا.