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Effect of Diazepam Therapy on Seizure Duration and GABAAα1-6 Receptors in Rats

1. Marsintauli Hasudungan Siregar*

Doctoral Study Program Medical Science, Faculty ofMedicine, Universitas Brawijaya, Malang 65145, IndonesiaAndPediatric Department of Dr. Moch Ansari Saleh General Hospital, Banjarmasin, South Kalimantan70125, Indonesia

marsintauli@student.ub.ac.id

2. Nurdiana

Departemen of Pharmacology, Faculty of Medicine, Universitas Brawijaya, Jl. Veteran, Malang 65145, East java, Indonesia

3. FarhadBal'afif

Department of Neurosurgery, Faculty of Medicine, Universitas Brawijaya, Jl. Veteran, Malang 65145, East java, Indonesia

4. Susanthy Djajalaksana

Department of Pulmonology, Faculty of Medicine, Universitas Brawijaya, Jl. Veteran, Malang 65145, East java, Indonesia

***corresponding author**

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Abstract

Background: Seizures are an outcome of an imbalance between excitation and inhibition. GABA is the primary inhibitory neurotransmitter in the central nervous system, and it interacts through GABAA receptors. The hippocampus is a crucial organ in seizure activity, as evidenced by studies that link changes in the expression of the GABAA receptor subunit to seizure activities.**Methods:**In this study, Wistar rats were split into two groups: one for treatment (diazepam 3 mg/kg BW) and the other for control (no therapy). PTZ (90 mg/kg BW) was used to induce status epilepticus. Thirty minutes after induction, the duration of the seizures was noted. GABAAα1-6 receptor expression was examined by immunohistochemistry in hippocampal brain tissues. One-way ANOVA and linear regression with a significance threshold of $p < 0.05$ were used to examine the data.**Result:**Seizures were considerably shorter when diazepam was used than when the control group was involved. The hippocampus's GABAAα1-6 receptor expression was investigated using immunohistochemistry, and the treatment and control groups were shown to differ considerably.**Conclusion:**The study found a correlation between seizure therapy and seizure duration but not between seizure therapy and GABAA receptors in the cortex, dentate gyrus, or cornu ammonis. IHK analysis showed similar expression in the GABAAα1-6 receptor region in seizure-affected animals, with treatment increasing in all areas. However, the dentate-gyrus region showed the highest expression of diazepam. Further research is needed to determine the appropriate dosage for seizure therapy.

Keywords: Diazepam Therapy, Seizure Duration, GABAAα1-6 Receptors

1. Introduction

Seizures are an indication of an imbalance between inhibition and excitation [1]. In the central nervous system, gamma-aminobutyric acid (GABA) is the most prevalent inhibitory neurotransmitter [2]. GABA signals, which mediate inhibitory signals, are received by GABAA receptors. Using immunohistochemical analysis, it is possible to determine the distribution of GABAA receptor subunits in distinct brain cell sites based on the subunit identity. In the cerebral cortex, hippocampus, striatum, cerebellum, amygdala, and brainstem, the GABAA receptor α1β2η2 subunit is most prevalent (60%), whereas the cerebellar area has the least amount of the α6βnγ2/δ subunit (<5%) [3].

GABA has a molecular mass of 103.12 g/mol, and its chemical formula is C3H2NO2. GABA receptors receive signals that are GABA-mediated. GABAA, GABAB, and GABAC receptors are the three different types of GABA receptors. GABAA and GABAB receptors are the only two subtypes of GABA receptors that are currently recognized; nevertheless, GABAC receptors are included in the GABAA receptor subunit group[4]. GABA receptors come in two different varieties based on their nature: the metabotropic GABAB receptor, which is a heterodimer, is metabotropic and has K^+ and Ca^{2+} channels via G protein, while the ionotropic pentameric GABAA receptor with Cl-channels is the second variety [2].Each subunit combination that makes up a GABAA receptor is unique. The human GABAA receptor, which has 19 subunits totalsix types of α subunits, three types of β subunits, three types of γ subunits, one type of δ subunit, one type of ε subunit, one type of π subunit, one type of θ subunit, and three types of ρ subunits[5].

GABA binding locations are depicted in two different charts above. Electrophysiological and pharmacological characteristics vary among subtypes[6]. Since each receptor subtype's function varies depending on brain cell activity, the electrophysiology of GABAA is also unique. Subunit makeup, ion levels in the environment, and GABA exposure all affect the conductance and time course of GABA receptor impulses. GABAA receptor physiologic processes are facilitated by the distribution, abundance, and differential regulation of GABAA receptor subtypes, which enable precise modulation and response across the brain[7]

The brain's GABA signaling pathway travels via the olfactory bulb, amygdala, hippocampus, hypothalamus, prefrontal cortex, spinal cord, and even the retina[4].GABA receptors were originally restricted to the postsynaptic membrane, but more recent studies have revealed that they are also present in dendrites and other tissues serving the same purpose [8]. Numerous CNS regions, including the frontal and occipital cortex, the hippocampus, and the cerebellum, have GABA receptors. [7].

Hippocampal region, which is composed of several pyramidal-shaped cells in each cornu ammonis (CA) section. The areas CA1, CA2, CA3, and CA4 comprise the hippocampus cornu ammonis[9]. The largest section, CA1, is delimited medially by CA2 and laterally by the presubiculum. Ninety percent of CA1 neurons are pyramidal cells, or glutamatergic neurons, with the remaining ten percent being interneurons. The dentate gyrus, which is bordered medially by the CA3 layer and laterally by the CA1 layer, contains the CA2 layer[10]. The supramammillary region of the hypothalamus provides input to CA2 of the cornu ammonis, but the dentate gyrus provides less input. The CA2 layer borders the CA3 layer medially and extends to the dentate gyrus's hilus. The CA3 layer's outermost portion is known as C4 [11]. The largest portion of pyramidal cells is called the CA3 region. While CA3 is sensitive to the effects of physical stress and exposure to chronic stress, pyramidal cells in the CA1 and CA2 regions are more sensitive to hypoxia [12].

2. Materials and Methods

2.1 Treatment of Experimental Animal

The research used 12 Wistar rats certified by the Food Security and Agriculture Service of the Bandung City Government. Rat underwent an acclimatization period of 7 days with standard feed and mineral water ad libitum, placed in clean cages every day according to laboratory standards. The inclusion criteria were male rat aged 8–9 weeks with a body weight of 180–240 grams. Exclusion criteria included illness during acclimatization, reduced food intake of more than 50%, dull or falling fur, weight loss of more than 10% post-adaptation, and death within 5 minutes after PTZ injection. The drop-out criterion is death before 24 hours after PTZ injection. Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Brawijaya University, with No. 825/EC/KEPK-S3/09/2023.

2.2Development of a Status Epilepticus Rat Model

Two groups of epileptic model rats were used in the experiment: control group, the control group, received no antiepileptic therapy, and treatment group, the treatment group, received diazepam 3 mg/kg body weight (BW). Pentylenetetrazole (PTZ) was given intraperitoneally at a dose of 90 mg/kg BW to produce status epilepticus. Five minutes after induction, behavioral monitoring was carried out to confirm status epilepticus, which is typified by jumping, tonic-clonic seizures, frenzied running, and falling (Racine score 5). Five minutes after the production of seizures, the experimental group received an injection of diazepam, while the control group received an injection of 2 ml NaCl 0.9%.

2.3Measurement of Seizure Duration

The rats were observed for 30 minutes following the induction of seizures with CCTV and a stopwatch to determine the duration of the seizures. The duration, which was measured in seconds, was the interval between the PTZ injection and the end of the seizure. Rats were monitored for twenty-four hours following the injection of either diazepam or NaCl. Once the 24-hour observation period had passed, all of the rats were killed, and their brains were taken out for further analysis.

2.4Measurement of GABAAα1-6 Receptors

The GABAA receptor was measured in hippocampal brain regions, especially in the region between C1 and C3. Brain tissue was sectioned into coronal slices 2-3 mm thick after being fixed for 7 hours with 10% formalin. The Thermo Scientific STP 120 tissue processor was used to prepare these sections. Santa Cruz Biotechnology's GABAARα1-6 (E-8) test kit was used for immunohistochemistry. The procedure included deparaffinization, quenching endogenous peroxidase, antigen unmasking, blocking serum albumin, incubating primary and secondary antibodies, applying SA-HRP and DAB substrate, mounting, and drying. Immunohistochemically marked GABAAα1-6 receptors in the hippocampus slices were identified by means of microscopic examination.

2.5Statistical Analysis

Data analysis was performed using SPSS version 25.0. A one-way Anova test was used to compare means between the control and treatment groups, while linear regression analysis was conducted to determine the relationship between the treatment and outcomes. The significance level was set at $p < 0.05$, indicating the threshold for statistical significance in the observed differences and relationships.

3. Result

Table 1 presents the basic data from six rats that were given a dose of 3 mg/kg BW of diazepam and six mice that were not given any medication. The mice's seizures were monitored for 30 minutes, and three areas were examined using immunohistochemistry/IHK: the dentate gyrus (GD), the cortex, and the Cornu ammonis 1 to 3 (CA1-3).

Table 1. Basic data on duration of seizures and IHK examination in seizure rats

No	Group	Seizure duration (seconds)	GD (%)	CA123 (%)	Cortex (%)
1	Control 1	148	8.40	0.79	0.88
2	Control 2	54	79.98	73.46	62.01
3	Control 3	122	5.93	7.57	4.27
4	Control 4	551	31.46	36.39	29.51
5	Control 5	104	30.44	18.43	22.63
6	Control 6	135	4.84	5.57	9.66
7	Treatment 1	14	23.54	36.02	22.00
8	Treatment 2	19	30.08	24.85	48.13
9	Treatment 3	152	83.14	80.53	58.35
10	Treatment 4	37	38.83	28.82	12.24
11	Treatment 5	21	77.64	51.29	10.27
12	Treatment 6	12	30.24	27.65	18.91

Descriptions: Control group = group excluded from therapy, specifically a group of status epilepticus model rats; Treatment Group (group of status epilepticus model mice administered diazepam 3 mg/kg BW).Cornu ammonis 1 to 3 (CA1-3); dentate gyrus (GD).

The findings of the two groups' immunohistochemistry analysis. Figure 1 shows the six rats in the control group (1 to 6). Figure 2 shows the six rats that make up the diazepam therapy group, or Treatment group, which is comprised of 1 through6.

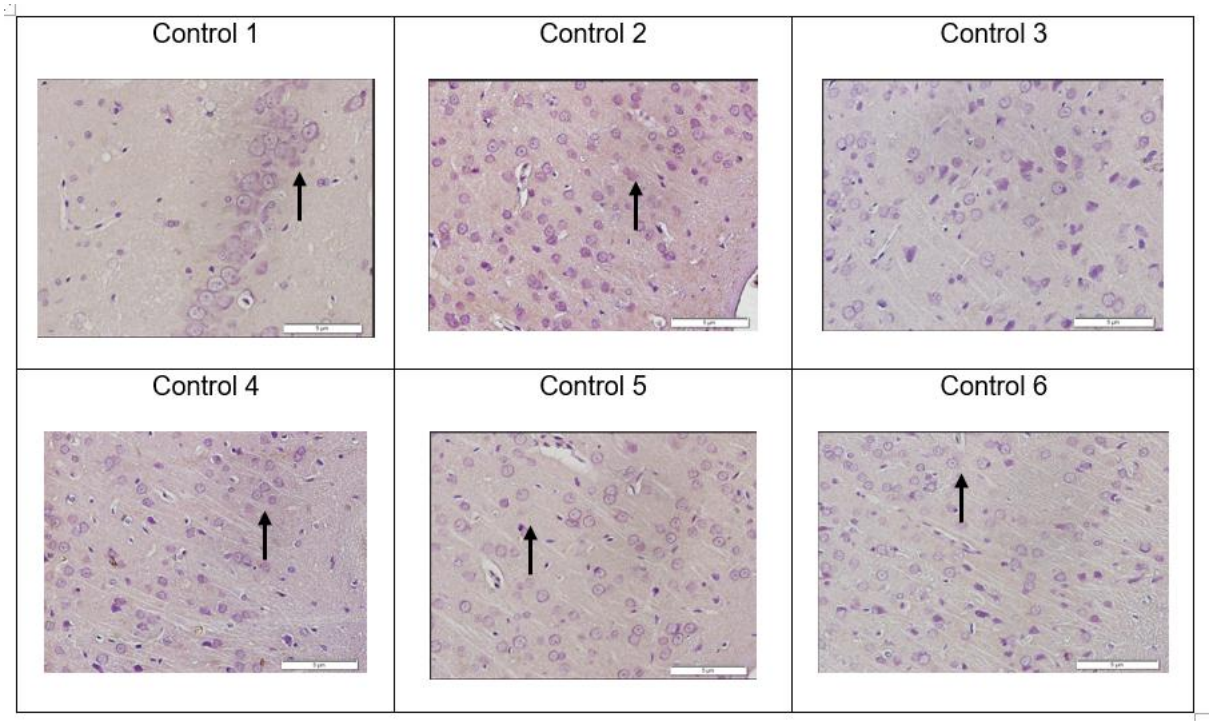

Figure 1: Rat IHK analysis with a control group Seizures induced by pentylenetetrazol
Description: An immunohistochemical technique using polyclonal anti-GABAAα1-6 was observed using a light microscope with 100x magnification. Arrows indicate GABA receptor α1-6 expression. control group

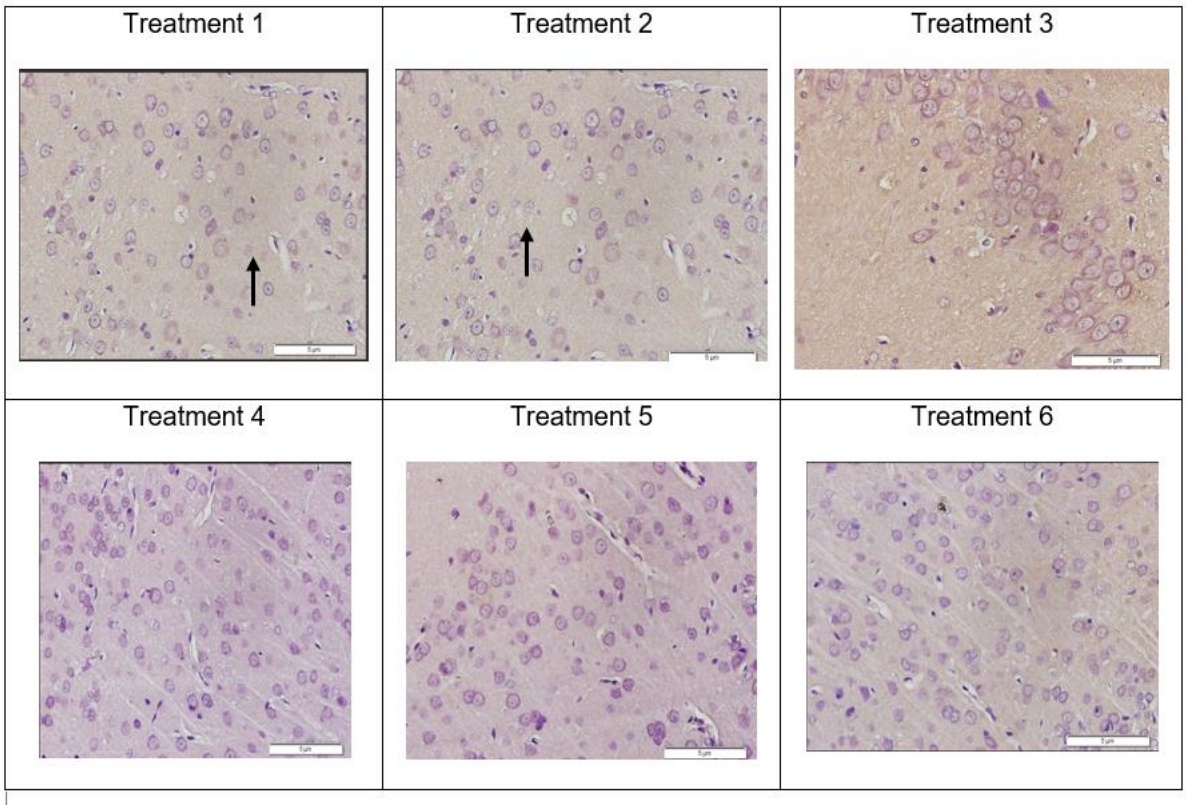

Figure 2. IHK examination in mice with convulsions induced by pentylenetetrazol in the diazepamtherapygroup
Description: An immunohistochemical technique using polyclonal anti-GABAAα1-6 was observed using a light microscope with 100x magnification. Arrows indicate GABA receptor α1-6 expression. Treatment group: therapy group

A comparison of seizure therapy-treated rats with control rats is shown in Table 2. As opposed to 185.67 seconds in the control group, the therapy group's mean seizure duration was 42.50 seconds.

Table 2. Descriptive data on seizure duration and IHK examination

Variable	Group	Mean (second)	SD	Minimum	Maxsimum
long seizure	Control group	185,67	181,941	54	551
	Treatment group	42,50	54,365	12	152
DG	Control group	26,8417	28,7034	4,84	79,98
	Treatment group	47,245	26,18722	23,54	83,14
CA123	Control group	23,7017	27,49039	0,79	73,46
	Treatment group	41,5267	21,34965	24,85	80,53
Cortex	Control group	21,4933	22,66601	0,88	62,01
	Treatment group	28,3167	20,455	10,27	58,38

Descriptions: control group: group excluded from therapy, specifically a group of status epilepticus model rats. Treatment Group: group of status epilepticus model rats administered diazepam 3 mg/kg BW.Cornu ammonis 1 to 3 (CA1-3); dentate gyrus (GD).

The IHK analysis of GABAA receptor α1-6 subunits in Figure 2 showed nearly identical results; all subunits (cortex by 28.3167%, dentate gyrus by 47.245%, and cornu

ammonis/CA123 by 41.5267%) had higher expression in the therapy group, although therapy had the highest expression. In cases of acute seizures, the dentate gyrus region contains diazepam. In contrast, the control group exhibited the lowest levels of GABAA receptor subunit α1-6 in the cornu ammonis/CA123 region.

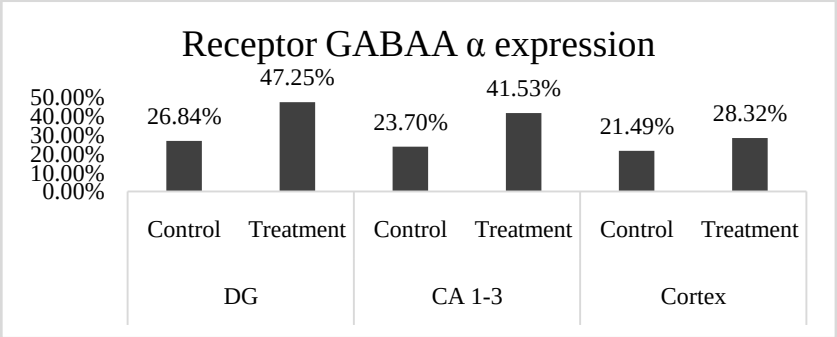

Figure 3. IHK examination in spastic rats
Description: control group group without therapy, i.e., group of status epilepticus model rats not receiving anticonvulsant therapy. Treatment Group: group of statusepilepticus model rats receiving diazepam 3 mg/kg BWCornu ammonis 1 to 3 (CA1-3); dentate gyrus (GD).

Table 3 shows that the diazepam group significantly affected the duration of seizures (p = 0.007*) and that the group's influence might be as much as 48% in the duration of seizures in a healthy rat (control). The GABAAα1-6 receptors in the dentate gyrus (p = 0.2), cornu ammonis (p = 0.238**), and cortex (p = 0.593) were not significantly affected by the diazepam group; nevertheless, the diazepam group was able to influence the dentate gyrus region (56%), cornu ammonis (50%), and cortex (67%).

Table 3. Hypothesis test of diazepam therapy in spastic rats

Variable		**Group**	**Mean (SD)**	**Significance Value**	**Mean Difference (IK 95%)**	**Determination Coefficient**
long seizure		Control	2,1463	0.007*	0,24093-1,19281	0,48
		Diazepam	1,4294			
Brain area	DG	Control	1,50	0,227	0,689- 1,795	0,56
		Diazepam	37,0433			
	CA1-3	Control	1,50	0,238**	0,701-1,801	0,50
		Diazepam	32,6142			
	Cortex	Control	1,50	0,593	0,835-1,949	0,67
		Diazepam	24,9050			

Description: • Cornu ammonis 1 to 3 (CA1-3); dentate gyrus (GD).

4. Discussion

In this research, 90 mg/kgBW of PTZ was utilized to elicit more severe seizures in the experimental rat (scoring 5 Racine). In the control group, the duration of the seizures was

185.67 ± 181.941 seconds, and protection was 100%. Meanwhile, in Oriaifo et al. (2012) research, rats were used in the experiment; PTZ was administered at a dose of 70 mg/kgBW (scoring 4 Racine) to cause seizures lasting 16.5 seconds, but it had no protective effect—all of the rats perished[13]. Comparably, experimental rat were given 90 mg/KgBW of PTZ to cause a seizure that lasted 528.6 seconds in a study by Vishwanath et al. (2012). No dead rats were discovered during the seizure[14]. The variations in the study's outcomes may be attributed to the fact that the strains and types of rats employed in each investigation, as well as their living conditions, may have affected the rat's resistance to seizure induction.

PTZ at a dose of 90 mg/kgBW was used to elicit more severe seizures in rat used in experimental animal studies (scoring 5 Racine). In the diazepam therapy group, 100% protection was maintained for seizures that lasted 42.50 seconds.Concurrently, in a study conducted by Vishwanath et al. (2012), laboratory ratwere given 90 mg/kg of PTZ to produce seizures, which lasted 65 seconds and protected every mouse. The rat also got 5 mg/kgBW of diazepam treatment. Experimental rat (scoring 4 Racine) were given PTZ at a dose of 70 mg/kgBW and 4 mg/kgBW of diazepam therapy in Chimbalkar et al. (2013). The seizures lasted for 51 seconds and did not produce any protection; as a result, all of the rat perished[15]. It may be concluded that seizure induction (dosage of pentylene tetrazole), the therapeutic dose of diazepam, and the rat's physical state all affect how long seizures last, despite the fact that the results of these experiments differ slightly from one another.

This investigation examined IHK in three different brain regions. In the dentate gyrus area (26.8417 ± 28.70%), cornu ammonis area (23.7017 ± 27.50%), and cortical area (21.4933 ± 22.67%), the control group's GABAA receptor expression was measured. In contrast, GABAA receptor expression was shown to be lower in other research. Thus, Zhang et al.'s study from 2021, which looked at GABAA receptor expression in the hippocampal area of mice given 90 mg/kg BW of pentylene tetrazole, revealed 17.46 ± 2.27% expression of these receptors. Jacob (2019) reports that GABAAα5 receptors were detected in 25% of cornu ammonis, which is consistent with hypothesis.

GABAA receptor expression was 49.86 ± 3. 32% in a study by Zhang et al. (2018) that looked just at IHK in the hippocampal area in mice that had seizures brought on by pentyelenetetrazole at a dose of 90 mg/kg BW and were given 5 mg/kg BW diazepam. In contrast to this study, which employed diazepam at a dose of 3 mg/kg BW, the expression of GABAA receptors was 41.53 ± 21.35 % in the dentate gyrus (DG) area, 41.53 ± 21.35 % in the cornu ammonis (CA) area, and 28.32 ± 20.46% in the cortical area[16]. This is consistent with the hypothesis that diazepam enhances GABAA receptor expression, which in turn increases GABAA receptor activity at larger dosages.

In this investigation, GABAA receptor expression in the dentate gyrus (p = 0.227), cornu ammonis (p = 0.238), and cortex (p = 0.593) did not significantly differ between diazepam therapy and controls. Unlike Zhang et al.'s research from 2018, there was a strong correlation (p = 0.001) between diazepam therapy and GABAA receptors in the hippocampal region when compared to controls[16]. The findings of this investigation contradict current understanding since diazepam functions as a GABA agonist, increasing the number of GABAA receptors. Diazepam's effect on GABAA receptor activity and the effect of other GABAA receptor subunits GABAA receptor binding are among the reasons. By binding to the interface at α (+) and γ (−), diazepam lowers excitation and many effects, including muscle relaxation, hypnosis, anxiolytics, and anti-epileptics. The effects of diazepam on GABAA receptors, which result in drug affinity effects, are mediated by several subtypes of GABAA receptors (Richter et al., 2020). In a case-control study of drug-resistant patients with temporal lobe epilepsy following epilepsy surgery, Sperk et al. (2021) examined anticonvulsant mechanisms and discovered alterations in the expression of GABAA receptor subunits α 4, α 5, and δ in the hippocampus region[17].

Diazepam dosages for dogs range from 0.5 to 2.0 mg/kg BW, whether administered intravenously, intranasally, or rectal [18]. The recommended intravenous dosage of diazepam is 0.15 to 0.2 mg/kg, with a maximum dose of 10 mg [19], [20]. It was discovered that the on diazepam dose was 6.2 in mice when a human dose of 0.5 mg/kgBW was converted using the conversion method (equivalent dose based on body surface area). Because diazepam was administered at a lower dose (3 mg), GABAA receptor activity was decreased, which resulted in low expression of GABAA receptors in this study.

5. Conclusion

In this study, there was a correlation between seizure therapy and the duration of the seizures (p = 0.007*), but not between seizure therapy and the GABAA receptors in the cortex (p = 0.593), dentate gyrus (p = 0.227), or cornu ammonis (p = 0.238). IHK analysis revealed nearly identical expression in the GABAAα1-6 receptor region in the brains of seizure-affected animals, with all three areas rising in the treatment group (DG by 47.245%, CA 123 by 41.5267%, and cortical by 28.3167%). But in the dentate gyrus region, where diazepam was used to treat acute seizures, the highest expression was seen (47.245%).

The α subunit plays a crucial role in determining the pharmacological characteristics of BZ; however, other GABAA receptor subunits, such as the γ or δ subunits, must also be examined as they may also have an impact on the pharmacological characteristics of anticonvulsants. Further research is also required to determine the appropriate dosage of

diazepam for seizure therapy.Accordingly, this study encourages additional research on the function of GABAA receptors in anti-seizure mechanisms, supporting the notion (Sperk et al., 2021) that GABAA receptors play a role a role in increased tonic inhibition, an anti-seizure mechanism.

6. Conflict of Interest

No conflict of interest

7. Author Contribution

All author contributed to the process of preparing this sytematic review article

8. References

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