Impact of cyclic nucleotides on the antiseizure activity of acute and chronic diazepam administration in isoniazid-induced seizures and pentylenetetrazole kindling in rats

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Background
It is estimated that ~10% of the population suffers a single convulsive episode during their lifetime. Epilepsy is the second most common chronic neurological disorder after stroke, affecting ~0.5% of the population in developed countries and 1.5–2% in developing countries. Diazepam (DZ) is among the benzodiazepines used most widely in status epilepticus as well as resistant and refractory seizures. However, tolerance to its antiseizure activity is among the obstacles facing it use on a wider basis. Exploration of the role of cyclic nucleotides in seizures might enable finding ways to combat tolerance as well as discover new treatment modalities.

Materials and methods
Seizure severity, electroencephalography, and levels of γ-aminobutyric acid (GABA), glutamate, cyclic adenosine monophosphate (cAMP), and cyclic guanosine monophosphate (cGMP) in brain homogenate were assessed upon single administration of isoniazid and repeated pentylenetetrazole injections, whether untreated or treated with DZ, either acutely or on a chronic basis, respectively.

Results
A single DZ injection, 10 min after single isoniazid, could significantly improve seizure severity, associated with increased power of the fast β wave, implying ameliorated cognitive functions, together with an equal GABA/glutamate ratio versus significant reduction in the cAMP/cGMP ratio. Repeated DZ injections, 10 min after each pentylenetetrazole injection, on alternate days for 1 month and every 2 days for another month produced a significant improvement in seizure severity, accompanied by reduced power of the fast β and the slow δ waves, associated with an increased GABA/glutamate ratio and an unchanged cAMP/cGMP ratio.

Conclusion
The current study assumes that a certain interplay exists between GABA/glutamate on the one hand and cAMP/cGMP on the other so that DZ could exert an anticonvulsant effect on an acute basis despite an unchanged GABA/glutamate ratio as well as upon chronic administration in the presence of an unchanged cAMP/cGMP ratio. In addition, the unchanged cAMP/cGMP ratio associated with chronic DZ administration suggests that cyclic nucleotides might lead to loss of some of the effects of DZ, as shown by reduced β wave power, indicating a reduction in cognitive abilities, concentration, and learning abilities, in contrast to higher β power after single use.

Keywords:
γ-aminobutyric acid, cyclic adenosine monophosphate, cyclic guanosine monophosphate, diazepam, epilepsy, glutamate, isoniazid, pentylenetetrazole, seizure

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Introduction

Seizure is an episode of abnormal neurologic function that can occur once [1]. Epilepsy is a tendency of occurrence of recurrent, spontaneous seizures [2].

In fact, epilepsy is known to be a disorder related to imbalance between the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and the excitatory glutamate. Metabotropic glutamate receptors (mGluRs) are presynaptic inhibitory autoreceptors linked to inhibition of adenyl cyclase (AC) and reduced cyclic adenosine monophosphate (cAMP). Conversely, GABA activates presynaptic autoinhibitory $GABA_B$ receptors, with subsequent suppression of AC and cAMP, leading also to inhibition of GABA release. Thus, cAMP can lead to inhibition of further GABA and glutamate release [3].

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There exist cyclic nucleotide-gated channels in the brain; one type is the nonselective cation channel linked to the metabotropic glutamate receptor, which, upon activation, causes stimulation of phosphodiesterase activity, leading to hydrolysis of cyclic guanosine monophosphate (cGMP), followed by closure of the cyclic nucleotide-gated channels and reduction in the response to glutamate [4].

It was found that a tonic cAMP-dependent kinase including protein kinase A inhibits a tonic $I_{k,}$ leading to global depolarization and a subsequent increase in neuronal excitability, together with enhanced cognitive performance [5].

Among the pharmacological agents commonly prescribed in Africa is diazepam (DZ) [6], acting as a $GABA_A$-positive allosteric modulator [7].

Despite the potency, rapid onset of action, and reasonable side-effect profile with short-term and long-term use of DZ, the development of tolerance as well as psychological and physical dependence limit their use [8].

Thus, the following study aims to clarify the role of cyclic nucleotides in the anticonvulsant effect of DZ on acute isoniazid (INH)-induced seizures and a pentylenetetrazole kindling model in rats.

Materials and methods

The experimental procedures were conducted at the Medical Pharmacology Department, Faculty of Medicine (Kasr Al-Aini), Cairo University, Egypt, 2012–2014. All experimental procedures were approved by the Medical Pharmacology Department and Faculty of Medicine Ethics committees. Fifty six adult male Sprague–Dawley rats, weighing 150–200 g, were purchased from the Laboratory Animal House Unit of the National Research Centre, Egypt, where they were housed in wire mesh cages at room temperature, 24 ± 2°C, with 30–70% relative humidity and a normal light/dark cycle of 12 h with a standard diet and free access to water ad libitum. Rats were acclimatized for 7 days before inclusion in the experimental procedures.

The animals were divided into three main groups: control (I), INH-induced seizures (II), and pentylenetetrazole kindling (III) groups. All groups were fasting and water deprived 24 h before administration of distilled water, diazepam [Memphis Company for Pharmaceuticals and Chemical Industries, Egypt, commercially available as Neuril ampoules (10 mg/2 ml) form, amber yellow oily solution], isoniazid (Sigma Aldrich, Germany, white powder freshly dissolved in distilled water on the day of the experiment), or pentylenetetrazole (Sigma Aldrich, Germany, white powder freshly dissolved in distilled water on the day of the experiment).

Group I, 'negative control groups' (n = 28), were subdivided into group IA (n = 14), healthy rats injected intraperitoneally with 10 mg/kg distilled water, and group IB (n = 14), healthy rats injected intraperitoneally with 10 mg/kg DZ [9], either once (n = 6) or repeatedly, every other day for 1 month, and then once, every other 2 days, for another month (n = 8).

INH-induced seizure model groups, Group II (n = 12) was subdivided equally into an untreated group 'Group IIA', healthy rats injected intraperitoneally with 250 mg/kg INH, once (n = 6), and a DZ-treated seizure model group 'Group IIB' (n = 6), rats injected intraperitoneally with 10 mg/kg DZ 10 min after a single intraperitoneal injection of 250 mg/kg INH.

Kindling model groups, 'Group III (n = 16)' was subdivided equally into an untreated group 'Group IIIA' (n = 8), pentylenetetrazole (PTZ) kindled rats injected intraperitoneally with 30 mg/kg PTZ once every other day for 1 month, and then once every other 2 days for another month, and DZ-treated kindled rats 'Group IIIB' (n = 8), PTZ kindled rats injected intraperitoneally with 10 mg/kg DZ, once, every other day for 1 month, and then once every other 2 days for another month. Each DZ injection was administered 10 min after intraperitoneal 30 mg/kg PTZ 'Modified from Giorgi *et al*. [10]'. In an attempt to compensate for deaths in all kindling models, higher numbers of rats were used.

Following drug(s) administration, after 15 min, an assessment was performed by video recording and analysis of rats' seizure severity for 2 h using Modified Racine Staging (0–6), together with measurement of both seizures' latency and duration in minutes [11].

Electroencephalography was recorded for 2 h under chloral hydrate anesthesia (intraperitoneally short of tachypnea) using ADInstruments PowerLab v.7.3.7, ADInstruments, Australia, followed by offline analysis of power spectral densities for amplitudes (μV^2/Hz) of source, β, α, θ, and δ waves [12], and illustrated with a standard PC-based hardware, Hp, windows v.7, USA.

The study took 2 months for the INH-induced seizure model groups, whether treated or not and related negative controls (2 weeks/group), and 4 months for PTZ kindling model groups, treated or not, and related negative controls (4 weeks/group). After completion of behavioral and EEG recording, rats were euthanized

by a sharp cut on the neck and then the brains were rapidly dissected, weighed, and stored in −80°C in Medical Biochemistry and Molecular Biology Department, Kasr Al-Aini, Cairo University. Brain homogenates were centrifuged and analyzed using the enzyme-linked immunosorbent assay technique for the measurement of brain levels of GABA (Bioassay Technology Laboratory, China), glutamate (CusaBio, China), cAMP (Bioassay Technology Laboratory), and cGMP (Bioassay Technology Laboratory).

All results were expressed as mean ± SD. Comparison of quantitative data between individual study groups was carried out using the Student *t*-test for independent samples to compare two groups and the one-way analysis of variance test with a post-hoc multiple group comparison to compare more than two groups. The results were significant if *P* values up to 0.05 [13]. All statistical calculations were carried out using statistical package for the social science, v.15 for Microsoft Windows, IBM, USA. Graphs were generated using Microsoft Office Excel, v.7.

Results

Compared with the untreated INH-induced seizure model group (group IIA), a single intraperitoneal injection of 10 mg/kg DZ 10 min after intraperitoneal 250 mg/kg INH in adult male rats (group IIB), resulted in significantly reduced seizure severity ($P = 0.000$), with reduced latency (in min) ($P = 0.000$), but longer duration ($P = 0.000$) (Table 1), manifested on EEG as significantly increased power of the fast β wave ($P = 0.000$), indicating enhanced intellectual performance (Table 2 and Fig. 1), together with an unchanged brain GABA/glutamate ratio ($P = 1.000$), versus a significantly reduced brain cAMP/cGMP ratio ($P = 0.000$) (Table 3 and Fig. 2).

Compared with the untreated PTZ kindled rats (group IIIA), repeated intraperitoneal injections of 10 mg/kg DZ, 10 min after each intraperitoneal 30 mg/kg PTZ (group IIIB), led to a significant reduction in seizure severity ($P = 0.000$), with earlier onset ($P = 0.000$) and similar duration ($P = 1.000$)

Table 1 Effect of diazepam on seizure severity (Modified Racine Staging from 0 to 6), latency, and duration of seizures of an INH-induced seizure model in awake rats (mean ± SD)

Group	Control group (group IA)	Diazepam (single i.p. 10 mg/kg)	Isoniazid (single i.p. 250 mg/kg)	Diazepam [single i.p. 10 mg/kg DZ, 10 min after INH (single i.p. 250 mg/kg INH)]
Modified Racine Staging for seizure severity (mean ± SD)	1	0[u]	3 ± 1.27[u]	0*
Latency	2.83 ± 4.4	0	14.5 ± 10.71	0*
Duration	6.5 ± 2.81	120[u]	4.67 ± 7.57	120*

DZ, diazepam; INH, isoniazid; i.p., intraperitoneal; u = significant in comparison to control group; *significant in comparison to INH group.

Table 2 Effect of diazepam on power spectral density (amplitude^2/frequency) (μV^2/Hz) of source wave, β, α, θ, and δ waves of an INH-induced seizure model (mean ± SD)

Group	Control	Diazepam (10 mg/kg, i.p.)	INH (250 mg/kg, i.p.)	Diazepam (10 mg/kg, i.p.), 10 min after INH (250 mg/kg, i.p.)
Source wave	$44.04^2/22.79 = 85.18 \pm 2.93$	$245.45^2/49.64 = 1213.81 \pm 16.57$[u]	$48.71^2/22.97 = 103.93 \pm 11.1$	$76.4^2/19.46 = 302.3 \pm 33.13$*
β wave	$33.9^2/27.15 = 42.49 \pm 4.52$	$244.99^2/49.97 = 1201.19 \pm 17.12$[u]	$40.41^2/28.46 = 57.61 \pm 5.51$	$52.68^2/20.64 = 134.86 \pm 10$*
α wave	$14.63^2/7.99 = 26.85 \pm 2.84$	$7.14^2/8.22 = 6.3 \pm 1.84$[u]	$10.46^2/7.73 = 14.64 \pm 5.58$[u]	$11.08^2/8.19 = 15.32 \pm 5.24$
θ wave	$6.39^2/4.74 = 8.82 \pm 2.61$	$7.25^2/4.5 = 11.89 \pm 4.14$	$13.28^2/4.9 = 36.7 \pm 10.24$[u]	$12.54^2/(4.96 = 32.63 \pm 11.32$
δ wave	$3.21^2/2.11 = 5.84 \pm 3.57$	$20.71^2/1.4 = 344.17 \pm 124.87$[u]	$9.94^2/2.29 = 45.2 \pm 15.38$	$6.77^2/2.32 = 21.27 \pm 9.48$

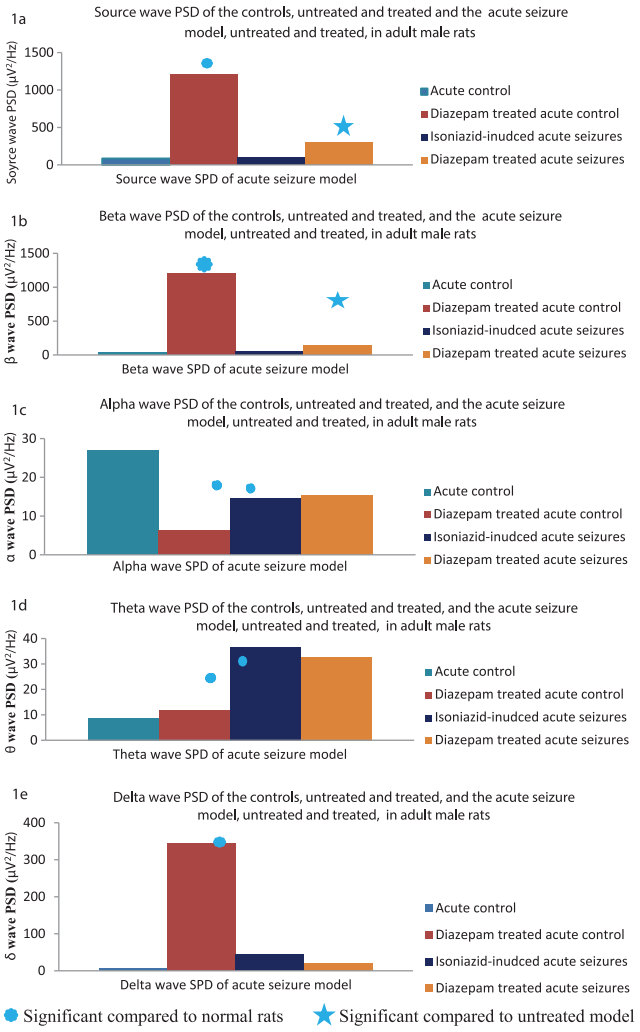
INH, isoniazid; i.p., intraperitoneally; u = significant in comparison to control group; *significant in comparison to INH group.

Table 3 Effect of diazepam on brain homogenate biochemical parameters (cAMP, cGMP, GABA, and glutamate) (μg/2 ml brain homogenate) of an INH-induced seizure model (mean ± SD)

Biochemical parameter	Control	Diazepam (10 mg/kg, i.p.)	INH (250 mg/kg, i.p.)	Diazepam (10 mg/kg, i.p., 10 min after INH (250 mg/kg, i.p.)
cAMP	15.83 ± 0.87	16.52 ± 0.51	19.97 ± 0.77[u]	10.11 ± 0.74*
cGMP	6.13 ± 0.58	6.92 ± 1.04	5.01 ± 0.94	4.11 ± 0.56
cAMP/cGMP	2.61 ± 0.33	2.43 ± 0.37	4.11 ± 0.77[u]	2.51 ± 0.5*
GABA	71.74 ± 0.73	70.14 ± 1.89	19.83 ± 0.84[u]	19.71 ± 1.46
Glutamate	845.29 ± 0.89	867.22 ± 1.71[u]	543.88 ± 1.72[u]	527.44 ± 0.85*
GABA/glutamate	0.09	0.08	0.04[u]	0.04

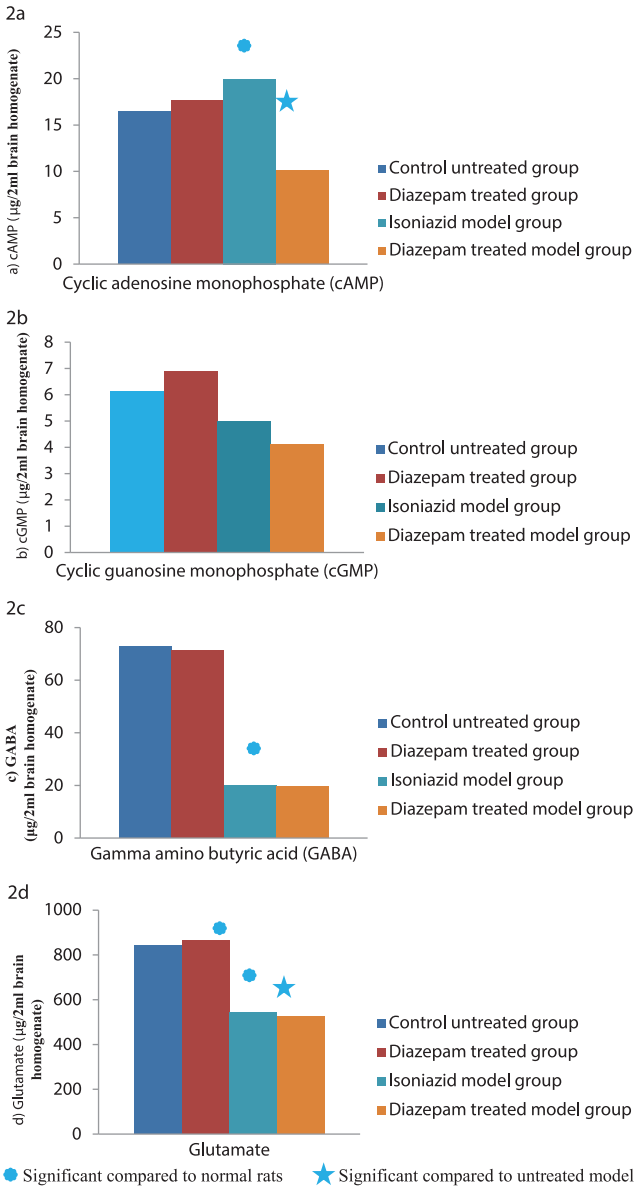
cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; GABA, γ-aminobutyric acid; INH, isoniazid; i.p., intraperitoneally; u = significant in comparison to control group; *significant in comparison to INH group.

Figure 1



Effect of diazepam (single i.p. 10 mg/kg, 10 min after i.p. 250 mg/kg INH) on PSD of (a) source wave, (b) β wave, (c) α wave, (d) θ wave, and (e) δ wave ($\mu V/Hz^2$) in anesthesized rats (chloral hydrate 50 mgl/kg) (mean ± SD). i.p., intraperitoneal.

Figure 2



Effect of diazepam (single i.p. 10 mg/kg, 10 min after i.p. 250 mg/kg INH) on brain homogenate biochemical parameters (a) cAMP, (b) cGMP, (c) GABA, and (d) glutamate (μg/2 ml brain homogenate) of rats (mean±SD). cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; GABA, γ-aminobutyric acid; INH, isoniazid; i.p., intraperitoneal.

(Table 4), together with EEG, indicating reduced β and δ power (P = 0.000) (Table 5 and Fig. 3), associated with a significant increase in the brain GABA/glutamate ratio (P = 0.000), whereas the brain cAMP/cGMP ratio was unchanged (P = 1.000) (Table 6 and Fig. 4).

Compared with normal rats, a single intraperitoneal DZ injection at a dose of 10 mg/kg resulted in improved seizure severity (P = 0.000), with similar latency (P = 0.679) but longer duration (P = 0.000) (Table 1), significantly increased β and δ power (P = 0.000), versus reduced α power (P = 0.000) (Table 2 and Fig. 1), together with unchanged brain GABA/glutamate (P = 0.091) and cAMP/cGMP ratios (P = 1.000) (Table 3 and Fig. 2).

Compared with normal controls, repeated DZ injections on alternate days for 2 months resulted in similar behavior, onset, and duration (P = 1.000) (Table 4), together with underlying significantly reduced β power (P = 0.000) against elevated θ power (P = 0.002) (Table 5 and Fig. 3). These changes were reflected biochemically as a reduction in both brain GABA/glutamate (P = 0.000) and cAMP/cGMP ratios (P = 0.000) (Table 6 and Fig. 4).

Discussion

In the current work, a single 10 mg/kg DZ injection reversed the INH-induced cAMP increase and

Table 4 Effect of diazepam on seizure severity (Modified Racine Staging from 0 to 6), latency, and duration of seizures of a PTZ kindling model in awake rats (mean ± SD)

Group	Control	Diazepam (i.p. 10 mg/kg, once every other day for 1 month, then once every other 2 days for another month)	PTZ (i.p. 30 mg/kg, once every other day for 1 month, then once every other 2 days for another month)	Diazepam [i.p. 10 mg/kg DZ, 10 min after PTZ (i.p. 30 mg/kg), once every other day for 1 month, then once every other 2 days for another month]
Modified Racine Staging for seizure severity (mean ± SD)	0	0	4.88 ± 0.79[u]	1.38 ± 0.52*
Latency	0	0	4.94 ± 4.35	14.75 ± 8.08*
Total duration	120	120	6.75 ± 6.86	1.21 ± 1.04

DZ, diazepam; i.p., intraperitoneal; PTZ, pentylenetetrazole ; u = significant in comparison to control group; *significant in comparison to INH group.

Table 5 Effect of diazepam on power spectral density (amplitude^2/frequency) (μV^2/Hz) of source wave, β, α, θ, and δ waves of a PTZ kindling model (mean ± SD)

Power spectral density (mean ± SD)	Control	Diazepam (i.p. 10 mg/kg), once every other day for 1 month, then once every other 2 days for another month	PTZ kindling (i.p. 30 mg/kg), once every other day for 1 month, then once every other 2 days for another month	Diazepam (i.p. 10 mg/kg), 10 min after PTZ (i.p. 30 mg/kg), once every other day for 1 month, then once every other 2 days for another month
Source wave	70.54 ± 5	43.1 ± 3.63[u]	45.42 ± 4.23[u]	29.33 ± 3.09*
β wave	66.55 ± 4.46	16.37 ± 1.31[u]	38.18 ± 2.42[u]	18.14 ± 3.1*
α wave	6.03 ± 0.74	8 ± 3.85	2.19 ± 1.29[u]	2.76 ± 0.7
θ wave	11.93 ± 2.94	22.45 ± 8.21[u]	8.88 ± 1.79	5.03 ± 0.74
δ wave	13.11 ± 2.64	27.71 ± 15.15	41.72 ± 7.66[u]	1.58 ± 0.35*

i.p., intraperitoneal; PTZ, pentylenetetrazole; u = significant in comparison to control group; *significant in comparison to INH group.

Table 6 Effect of diazepam on brain homogenate biochemical parameters (cAMP, cGMP, GABA, and glutamate) (μg/g brain weight) of a PTZ kindling model in rats (mean ± SD)

Group	Control untreated group	Diazepam (i.p. 10 mg/kg), once every other day for 1 month, then once every other 2 days for another month	PTZ kindling (i.p. 30 mg/kg), once every other day for 1 month, then once every other 2 days for another month	Diazepam (i.p. 10 mg/kg), 10 min after PTZ (i.p. 30 mg/kg), once every other day for 1 month, then once every other 2 days for another month
cAMP	20.79 ± 1.76	6.65 ± 0.69[u]	27.86 ± 1.35[u]	23.2 ± 1.53*
cGMP	8.06 ± 1.44	6.9 ± 0.58	17.64 ± 1.26[u]	15.46 ± 1.58*
cAMP/cGMP	2.64 ± 0.4	1 ± 0.17[u]	1.59 ± 0.19[u]	1.51 ± 0.15
GABA	78.58 ± 1.23	54.82 ± 1.69[u]	124.88 ± 1.68[u]	113.4 ± 1.95*
Glutamate	570.54 ± 0.9	1448.93 ± 1.81[u]	801.33 ± 1.91[u]	97.44 ± 1.19*
GABA/glutamate	0.14	0.04[u]	0.16[u]	1.16 ± 0.03*

cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; GABA, γ-aminobutyric acid; i.p., intraperitoneal; PTZ, pentylenetetrazole; u = significant in comparison to the control untreated group; *significant in comparison to the PTZ kindling.

restored the imbalance between GABA and glutamate, by reducing glutamate, as was shown behaviorally by reduced seizure severity as well as increased β power, indicating enhanced cognitive abilities.

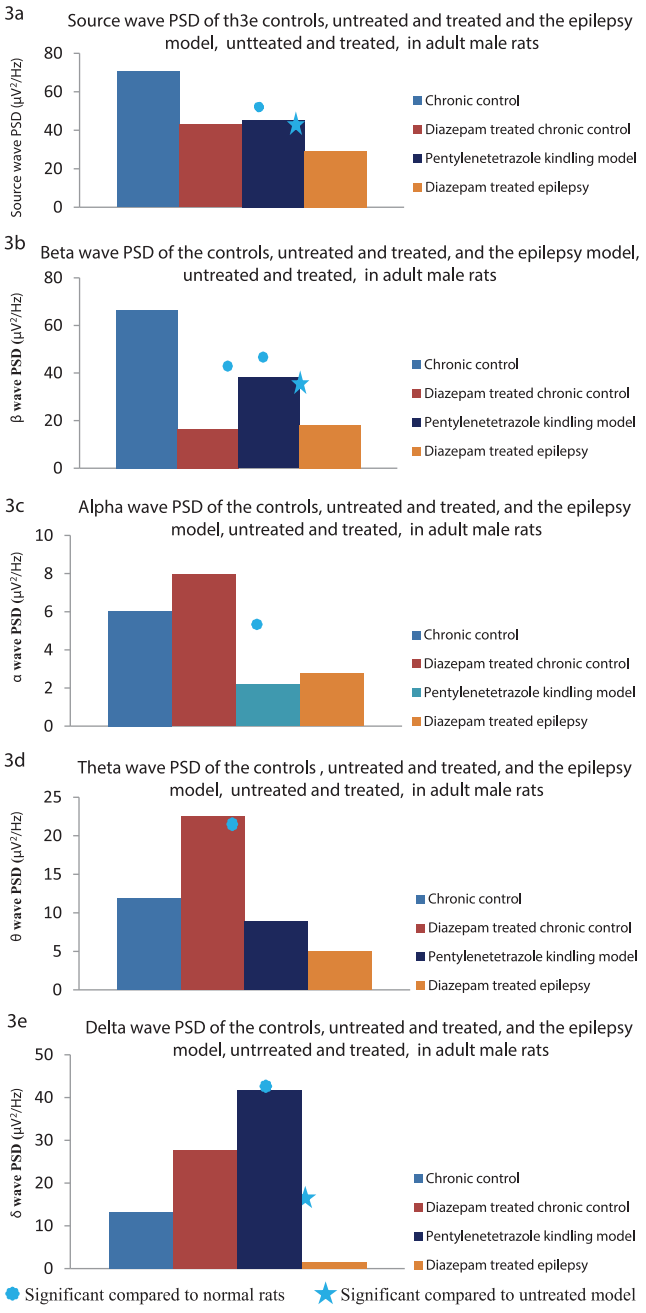
Similarly, Ying-Jun *et al.* [14] found these changes with the co-administration of DZ (0.1 g/l, 3 μl) and sodium-penicillin (1000 kIU/l, 3 μl), a $GABA_A$ receptor blocker and indirect glutamate releaser [15], through the intracerebroventricular route, in adult male Sprague–Dawley rats. However, prominent effects were observed on the low-frequency bands, θ and δ, more than the fast frequency band, β.

Crawford *et al.* [16] explained the link between cAMP and neuronal excitability by hypothesizing a depolarized state through a Go protein-regulated neuronal Ca^{2+} channel, with a subsequent increase in calcium levels and phosphorylation of intracellular proteins, responsible for increasing neuronal excitability [17].

In contrast to the unchanged brain GABA upon single DZ administration in the current research, Gandhimati and Kumar [18] reported a DZ-mediated increased GABA level upon oral intake of 4 mg/kg in male albino rats, although in two different models of rat-induced seizures (maximal electroshock and 90 mg/kg subcutaneous PTZ).

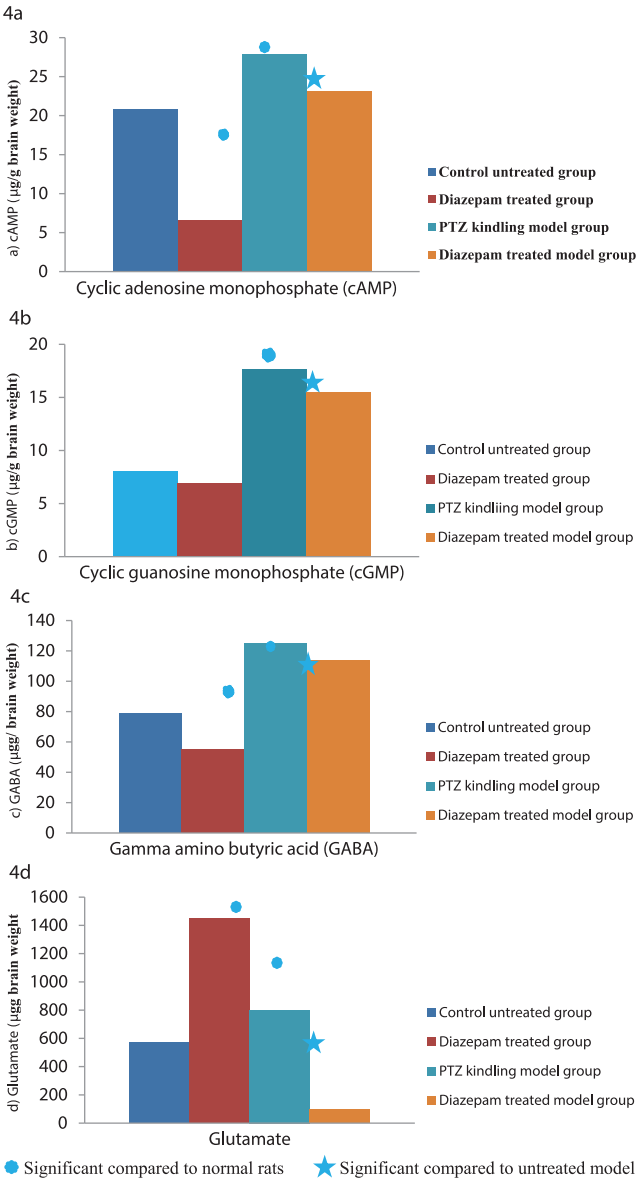
Comparison of the DZ-treated control group with the healthy group showed improved seizure scoring severity as DZ is known to suppress physiological

Figure 3



Effects of diazepam (challenge i.p. 10 mg/kg after 5 days of abstinence, following repeated dosing, once every other day for 1 month, then once every other 2 days for another month, each dosing 10 min after i.p. 30 mg/kg PTZ) on PSD of (a) source wave, (b) β wave, (c) α wave, (d) θ wave, and (e) δ wave (μV/Hz²) in anesthesized rats (chloral hydrate 50 mgl/kg) (mean ± SD). i.p., intraperitoneal, PSD, power spectral density; PTZ, pentylenetetrazole.

Figure 4



Effect of diazepam (challenge i.p. 10 mg/kg after 5 days of abstinence following repeated dosing, once every other day for 1 month, then once every other 2 days for another month, each dosing 10 min after i.p. 30 mg/kg PTZ) on brain homogenate biochemical parameters (a) cAMP, (b) cGMP, (c) GABA, and (d) glutamate in rats (mean ± SD). cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; GABA, γ-aminobutyric acid; i.p., intraperitoneal; PTZ, pentylenetetrazole.

tremors that might occur in healthy individuals [19] and are generally not associated with seizure activity. Enhanced physiological tremors may be amplified by anxiety or fear and are visible to the naked eye [20]. Besides, benzodiazepines are reported to cause intention tremors [21].

In agreement with the current investigation in which single DZ administration to normal rats led to decreased α activity compared with the untreated control group, similar results were reported by Basani *et al.* [22].

As in the present experimental work, a single DZ injection to normal rats induced a decrease in brain GABA in a trial focusing on the management of diisopropyl fluorophosphates-induced seizures (subcutaneously 1.5 mg/kg) [23] in adult male Fisher rats with $GABA_A$-positive allosteric modulators, imidazenil (intraperitoneally 0.5 mg/kg) and midazolam (intraperitoneally

0.5–2 mg/kg) [24]. However, an increase in glutamate was also detected. This can be attributed to a dual mechanism of benzodiazepines involving glutamatergic activation versus $GABA_A$ receptor inactivation [25].

In agreement the current study, Das and Guha [13], in their amygdala kindling rat model, reported that repeated intraperitoneal administration of 20 mg/kg DZ limited the seizure stage in Racine staging, together with abolished high-voltage spikes (slow δ wave), accompanied by a decrease in dopamine level with a subsequent decrease in cAMP signaling.

In the present work, a concomitant decrease in cAMP, together with decreased glutamate and improved seizure severity as well as reduced power of high-frequency (β) and high-voltage (δ) waves in DZ-treated epileptic rats could be attributed to the activation of presynaptic mGluRs, known to regulate glutamate release negatively [26]. mGluRs are coupled to Gi/Go proteins, with subsequent negative regulation of AC and voltage-sensitive Ca^{2+} channels [27].

In agreement with a DZ-associated cAMP decrease upon chronic administration in the present investigation, studies on adult male Sprague–Dawley rats found that DZ exerted differential effects on GABA subunits, ranging from increased α1 and γ2 subunits to decreased α3 subunit, causing downregulation of calcium/calmodulin protein kinase II, secondary to increased glutamate [28].

A proposal was made about excitatory GABAergic activity as a contributor to epilepsy [29] as this was implicated in postinjury seizures after traumatic brain injury [30].

Also, the decrease in GABA upon repeated DZ injections could be attributed to a decrease in $GABA_A$ receptor expression that was associated previously with resistance to the antiepileptic effect of phenobarbital in animal models [31].

Also, in cases of refractory epilepsy, GABA receptors are internalized together with externalization of AMPA receptors with decreased efficacy of GABA agonists [32] as in the case of DZ administration in status epilepticus [33].

In addition, dating to 1985, Marescaux *et al.* [34] reported a DZ antagonizing effect on GABAmimetics in rats with spontaneous petit mal-like epilepsy.

Conclusion

The current study assumes that cyclic nucleotides might be linked to the anticonvulsant effect of DZ in the treatment of acute INH-induced seizures.

However, this effect was lost upon chronic DZ administration, suggesting that cyclic nucleotides might play a role in tolerance to some of the effects of DZ, but not the anticonvulsant one.

With chronic DZ administration, deterioration in cognitive abilities could be attributed to consumption or downregulation of second messengers, including second messengers.

Future studies are recommended to explore the link between tolerance and cyclic nucleotides in other animal species, using other drugs causing dependence in other seizure models, together with assessment of learning and cognitive abilities.

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Conflicts of interest

There are no conflicts of interest.

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