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How Rolipram, as a Phosphodiesterase-4 Inhibitor, Affects Acute Isoniazid-Induced Seizures and Pentylenetetrazole Kindling in Rats

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Abstract

Background: It is estimated that 10% of the population suffer 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.

Aim: The target of this investigation was to find out the effect of phosphodiesterase-4 inhibitor (PDE4I), rolipram (Rol), on the isoniazid (INH)-induced seizures and the pentylenetetrazole (PTZ) kindled rats, in comparison to the benzodiazepine (BZ) agonist, diazepam (DZ).

Methods: Acute seizures were produced by intraperitoneal (i.p.) administration of INH, 250 mg/kg, either alone or 10 minutes before either i.p. 10mg/kg DZ, or i.p. 0.5mg/kg Rol. Kindling model was produced by repeated i.p. administration of 30mg/kg PTZ, every other day, for one month and every two days for another month, either alone or followed, ten minutes later, by i.p. 10mg/kg DZ or 0.5mg/kg Rol. Following drug(s) administration, seizure severity score, electroencephalography, biochemical measurement of gamma-aminobutyric acid (GABA), glutamate, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) in brain homogenate were done.

Results: In the acute INH-induced seizures, single i.p. 0.5 mg/kg Rol effects were opposite to DZ. These included unchanged seizure severity, significantly reduced power of the fast waves (ß and a), significantly increased brain GABA/glutamate ratio, together with no significant change in cAMP/cGMP ratio. While in kindled rats, i.p. 0.5mg/kg Rol administration on chronic basis, showed similar effects, but less than DZ, in the form of significantly improved seizure severity score, accompanied by reduced power of S wave with unchanged a and 0 waves, significantly increased GABA/glutamate ratio, together with unchanged cAMP/cGMP ratio.

Conclusion: Based on this preclinical study, caution with the use of rolipram in case of INH-induced seizures. In contrast to this preliminary warning, rolipram might be able, on chronic basis, to aid in the management of epilepsy, though less than DZ. These changes did not correspond to cyclic nucleotides changes.

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Key Words: *Isoniazid – Seizures – Pentylenetetrazole – Epilepsy – Diazepam – Rolipram – cAMP – cGMP – GABA – Glutamate.*

Introduction

SEIZURE OR ICTUS is an episode of abnormal neurologic function due to abnormal electrical discharge of neurons, which can occur once [1], while epilepsy is a brain disorder, characterized by unpredictable and periodic, transient alteration in behavior [2].

Isoniazid (INH) is widely used in treatment and chemoprophylaxis of tuberculosis. It inhibits pyridoxal phosphate (PLP), a necessary cofactor for glutamic acid decarboxylase (GAD), leading to inhibition of GABA synthesis [3]. The resultant toxic effect is drug-resistant generalized tonic-clonic seizures [4].

Chemical kindling model is a model with permanently lowered seizure threshold with spontaneous seizures [5], following repeated systemic injections of pentylenetetrazole (PTZ), the prototype agent of systemic convulsants [6] that act by blocking GABAergic system [7]. After a silent period of 1-3 weeks of neuronal degeneration and neurogenesis with synaptic reorganization, spontaneous seizures occur [8]. It is considered a model of human temporal lobe epilepsy [9].

Since their introduction in clinical practice, benzodiazepines were widely used as anticonvulsants, including diazepam, a high-efficacy, non-selective, positive allosteric modulator of GABAA receptors [10].

Despite huge funding for new antiepileptic drug development, many of them were withdrawn or restricted for use because their adverse effects outweigh their beneficial actions as hepatic failure, aplastic anemia and visual field defects [11]. Moreover, newly developed antiepileptic drugs are expensive to be afforded by patients of the third world countries [12].

Phosphodiesterases (PDEs) are metallophosphorylases enzymes that hydrolyze AMP and GMP [13]. Phosphodiesterase-4 is cAMP-specific that constitutes 70-80% of PDE activity in neuronal tissue [14].

Rolipram is especially important in inhibiting cAMP hydrolysis in neuronal tissues [15], with subsequent activation of cAMP-dependent protein kinase (PKA) together with phosphorylation and activation of cAMP-response element binding (CREB) proteins [16], being a critical signaling pathway in the regulation of adult neurogenesis [17].

Rolipram possesses distinct properties that might aid its long-term oral use [18] in epilepsy such as its preferential action in hippocampus [19], its low molecular weight with the ability to pass the blood-brain barrier [20] together with few unthreatening adverse effects [21].

Approaches in the treatment of epilepsy involve neuroprotection, anti-inflammation, immunosuppression and neuromodulation [22]. Rolipram was claimed to have a biphasic neuroregenerative property [23], secondary to increased intracellular cAMP [24] as well as anti-inflammatory potentials [25].

Accordingly, the current research work aims to explore the role of rolipram administration, compared to diazepam, in the course of both the isoniazid-induced seizure model and the pentylenetetrazole kindling model in rats and whether linked to brain cAMP/cGMP levels.

Material and Methods

Eighty four adult male Sprague-Dawley rats, weighing 150-200gm, were housed in wire mesh cages at room temperature 24±2°C with 30-70% relative humidity and normal light/dark cycle of 12 hours with standard diet and free access to water ad libitum. Rats were acclimatized for 7 days before inclusion into the experimental procedures in Medical Pharmacology Department, Faculty of Medicine (Kasr Al-Aini), Cairo University, Egypt, 2012-2014.

The animals were divided into three main groups. All groups were fasting and water deprived 24 hours before distilled water or pharmacological agents, diazepam (DZ), rolipram (Rol) intraperitoneal (i.p.) injections (control groups "I"), INH i.p. injections (INH-induced seizure model groups "Groups II") and PTZ i.p. injections (Kindling model groups "Groups III") were administered.

Groups I "Control groups" (n=42), they were subdivided into "group IA (n=14)", healthy rats injected i.p. with distilled water, "group IB DZ treated control group (n=14)" , healthy rats injected i.p. with 10mg/kg DZ, either once as in INH-induced seizure model groups [26] or repeatedly once every other day for one month, then once every other 2 days for another month as in PTZ kindling groups [27] and group IC "Rol treated control group", healthy rats injected i.p. with 0.5mg/kg Rol, either once or once every other day for one month, then once, every other two days, for another month [28].

Groups II "INH-induced seizure model groups" (n=18), subdivided equally into Group IIA "untreated model group", healthy rats injected i.p. with 250mg/kg INH, once, Group IIB "DZ treated model group", seized rats injected i.p. with 10mg/kg DZ, 10 min. after single i.p. 250 mg/kg INH and Group IIC "Rol treated model group", seized rats injected i.p. with 0.5mg/kg Rol, 10min. after single i.p. 250mg/kg INH [29].

Groups III "PTZ kindling model groups" (n=24), subdivided equally into Group IIIA "untreated model group, healthy rats injected i.p. with 30mg/kg PTZ, once every other day for one month, then once every other two days for another month, Group IIIB "DZ kindled rats", kindled rats injected i.p. with 10mg/kg DZ, once every other day for one month, then once every other two days for another month, each dosing 10min. after i.p. 30mg/kg PTZ and Group IIIC "Rol treated kindled rats", kindled rats injected i.p. with 0.5mg/kg Rol, once every other day for one month, then once every other two days for another month, each dosing 10min. after single i.p. 30mg/kg PTZ "Modified from Giorgi et al. [30]", who injected PTZ, daily for 30 days.

Isoniazid, pentylenetetrazole were obtained from (Sigma Aldrich) and rolipram from (Enzo Life Sciences). All three drugs were supplied as white powder, freshly dissolved in distilled water at the day of the experiment.

Diazepam was commercially available as Neuril® ampoules (10mg/2ml) from (Memphis

Company for Pharmaceuticals and Chemical Industries), as amber yellow oily solution.

Biochemical kits for ELISA assessment of brain levels of GABA, cAMP and cGMP were available from (Bioassay Technology Laboratory), while ELISA measurement of brain glutamate was done using biochemical kit supplied form (CusaBio).

Behavioral analysis:

Rats' seizure severity was scored for two hours using Modified Racine Staging (0-6), together with measurement of both seizures' latency and duration in minutes [31].

Electroencephalography:

Electroencephalography was recorded for two hours under chloral hydrate anesthesia (i.p. short of tachypnea) using (ADInstruments PowerLab v.7.3.7), followed by offline analysis of power spectral densities for amplitudes ($\mu V^2/Hz$) of source, beta (β), alpha (α), theta (θ) and delta (δ) waves [32] and illustrated with a standard PC-based hardware, windows v.7.

Biochemical study:

Rats were euthanized after completion of behavioral or EEG recording, then brains were rapidly dissected, weighed and stored in –80°C in Medical Biochemistry and Molecular Biology Department, Kasr Al-Aini, Cairo University for analysis of GABA, glutamate, cAMP and cGMP.

Statistical analysis:

All results were expressed as mean ± standard deviation (SD). Comparison of quantitative data between individual study groups was done using student *t*-test for independent samples in comparing two groups and one way analysis of variance (ANOVA) test with posthoc multiple group comparison in comparing more than two groups. Results were significant if p-values 0.05 [26]. All statistical calculations were done using Statistical Package for the Social Science (SPSS), v.15 for Microsoft Windows. Graphs were generated using Microsoft Office Excel, v.7.

Results

Results of isoniazid-induced seizures:

Compared to the untreated INH-induced seizure model group (group II), single i.p. injection of 0.5mg/kg RoI, ten minutes after i.p. 250mg/kg INH in adult male rats (group IIIB), resulted in unchanged seizure severity score, with reduced latency and longer duration, in contrast to DZ (group IIIA) which showed significant improvement in seizure severity (Tables 1,2).

Also in relation the INH seizure model group (II), EEG of RoI (IIIB) manifested reduced power of fast waves (β and α), in contrast to the effects of DZ (Table 3, Fig. 2).

In relation to the model group (II), RoI (IIIB) resulted in significantly increased brain GABA/glutamate ratio, with no corresponding change in cAMP/cGMP ratio. These variations were contradictory to DZ treated seizure (group IIIA) (Table 4, Fig. 3).

Compared to the control groups, whether untreated or DZ-treated (groups IA and IB, respectively), single i.p. RoI injection at a dose of 0.5mg/kg (group IC) did not change seizure severity, but resulted in earlier onset and longer duration (Tables 1, 2), accompanied by significant increases in β, θ and δ power versus reduced α power (Table 3, Fig. 2), together with reduced GABA/glutamate ratio and an underlying higher brain cAMP. These RoI-induced variations were in contrast to DZ (group IB) (Table 4, Fig. 3).

Table (1): Effects of rolipram, compared to diazepam, in control and INH-induced seizure model on seizure severity (modified Racine staging from 0 to 6) in awake rats (mean±SD).

Group	Control group (group IA)	Diazepam treated control (group IB) [Single i.p. 10mg/kg]	Rolipram treated control (group IC) [Single i.p. 0.5 mg/kg]	Isoniazid-induced seizure model group (group II) [Single i.p. 250 mg/kg]	Diazepam treated model group (group IIIA) [Single i.p. 10 mg/kg DZ, 10 minutes after single i.p. 250 mg/kg INH]	Rolipram-treated model group (group IIIB) [Single i.p. 0.5 mg/kg DZ, 10 minutes after single i.p. 250 mg/kg INH]
Modified Racine staging for seizure severity (Mean±SD)	1	0	1.33±1.03	3±1.27*	0✱	2.08±1.2

* Significant compared to control group.
✱ Significant compared to isoniazid-induced seizure model group.

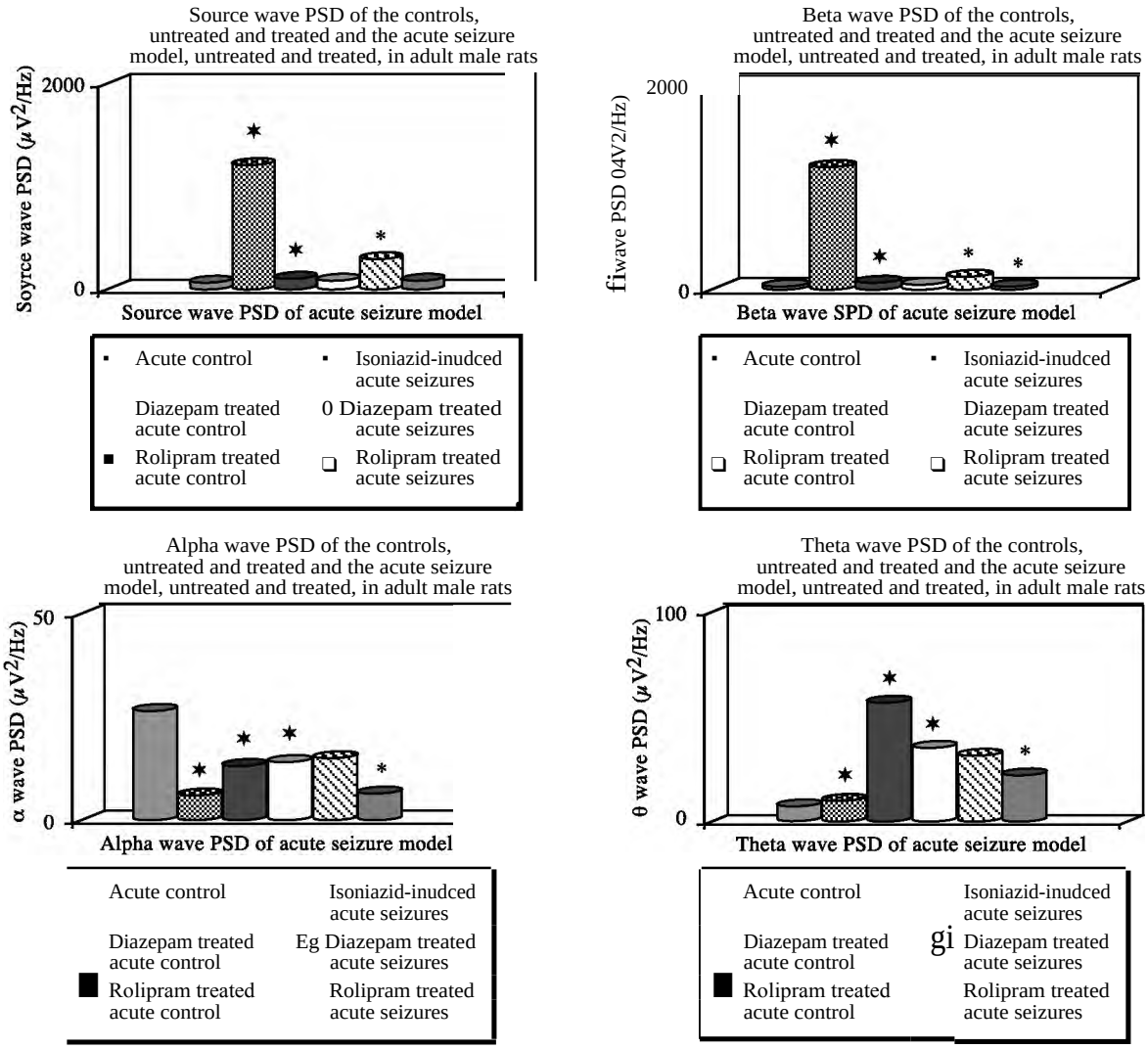
Table (2): Effects of rolipram, compared to diazepam, in control and INH-induced seizure model on latency (min.) and duration (min.) in awake rats (mean±SD).

Peak Seizures	Control group (group IA)	Diazepam treated control (group IB) [Single i.p. 10 mg/kg]	Rolipram treated control (group IC) [Single i.p. 0.5 mg/kg]	Isoniazid-induced seizure model group (group II) [Single i.p. 250 mg/kg]	Diazepam treated model group (group IIIA) [Single i.p. 10 mg/kg DZ, 10 minutes after single i.p. 250 mg/kg INH]	Rolipram-treated model group (group **IIIB)** [Single i.p. 0.5 mg/kg, 10 minutes before single i.p. 250 mg/kg INH]
Latency	2.83±4.4	0	1.67±1.75	14.5±10.71	0	3.71±4.28
Duration	6 .5±2.81	120	20±22.24	4.67±7.57	120	54.18±59.52

Table (3): Effects of rolipram, compared to diazepam, in control and INH-induced seizure model on power spectral density (PSD) [amplitude^2/frequency] V^2/Hz) of source wave, beta, alpha, theta and delta waves in anesthesized rats (chloral hydrate) (mean±SD).

Power spectral density (PSD) [Mean ± SD]	Control untreated group	Diazepam treated group (10 mg/kg, i.p.)	Rolipram treated group (0.5 mg/kg, i.p.)	Acute INH-induced seizure model group (250 mg/kg, i.p.)	Diazepam treated seizure model group (10 mg/kg, i.p., 10 min. after INH)	Rolipram treated seizure model group (0.5 mg/kg, i.p., 10 min. after INH)
Source Wave	85.18±2.93	1213.81±16.57*	120.21±8.61*0	103.93±11.1	302.3±33.13*	87.09±11.05
Beta Wave	42.49±4.52	1201.19±17.12*	62.76±6.38*	57 .61±5 .51	134.86±10*	39.18±3.69*
Alpha Wave	26.85±2.84	6.3±1.84*	13.67±2.23*G	14.64±5.58*	15.32±5.24	6.99±1.54*
Theta Wave	8.82±2.61	11.89±4.14	58.19±11.63*0	36 .7±10 .24*	32.63±11.32	23.5±5.2
Delta Wave	5.84±3.57	344.17±124.87*	360.26±59.88*	45.2±15.38	21.27±9.48	141.18±37.2

* Significant compared to control group. ()Significant compared to diazepam treated group.
* Significant compared to isoniazid-induced seizure model group.



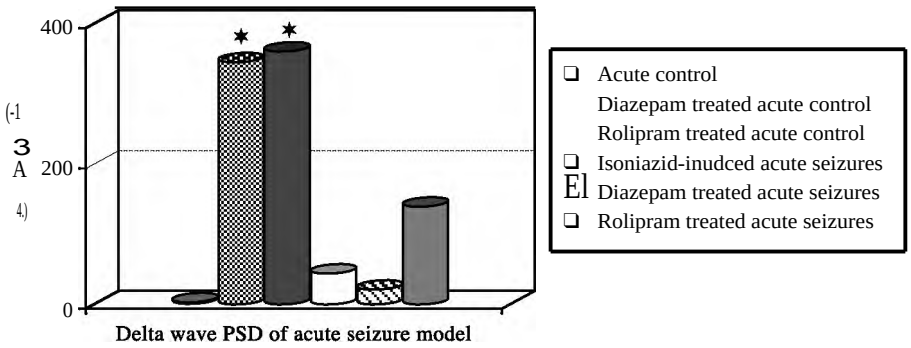


Fig. (1): Effects of rolipram (single i.p. 0.5 mg/kg, 10 min. after i.p. 250 mg/kg INH), compared to diazepam (single i.p. 10 mg/kg, 10 min. after i.p. 250 mg/kg INH) on PSD of (a) Source wave, (b) Beta wave, (c) Alpha wave, (d) Theta wave and (e) Delta wave 04V/Hz) in anesthesized rat (chloral hydrate 50 mg/kg) (mean±SD).

Table (4): Effects of rolipram, compared to diazepam, in control and INH-induced seizure model on brain homogenate biochemical parameters (cAMP, cGMP, GABA and glutamate) 0.4g/ 2 ml brain homogenate) in rats (mean±SD)

Biochemical parameter	Control untreated group	Diazepam treated control group (10 mg/kg, i.p.)	Rolipram treated control group (0.5 mg/kg, i.p.)	Acute INH-induced seizure model group (250 mg/kg, i.p.)	Diazepam treated seizure model group (10 mg/kg, i.p., 10 min. after INH)	Rolipram treated seizure model group (0.5 mg/kg, i.p., 10 min. after INH)
cAMP	15.83±0.87	16.52±051	19.83±1.39*	19.97±0.77*	10.11±0.74*	13.48±0.32* •
cGMP	6.13±058	6.92±1.04	5.7±0.7	5.01±0.94	4.11±0.56	2.7±0.33*
cAMP/cGMP	2.61±0.33	2.43±0.37	3.54±0.62	4.11±0.77*	2.51±0.5 *	5.06±0.7*
GABA	71.74±0.73	70.14±1.89	31.68±1.09*	19.83±0.84*	19.71±1.46	31.75±1.45*
Glutamate	845.29±0.89	867.22±1.71*	1189.18±1.31*0	543.88±1.72*	527.44±0.85*	554.42±2.22*
GABA/glutamate	0.09	0.08	0.03*	0.04*	0.04	0.06*

*Significant compared to control untreated group.
OSignificant compared to diazepam treated control group.
* Significant compared to isoniazid-induced seizure model group.
* Significant compared to diazepam treated seizure model group.

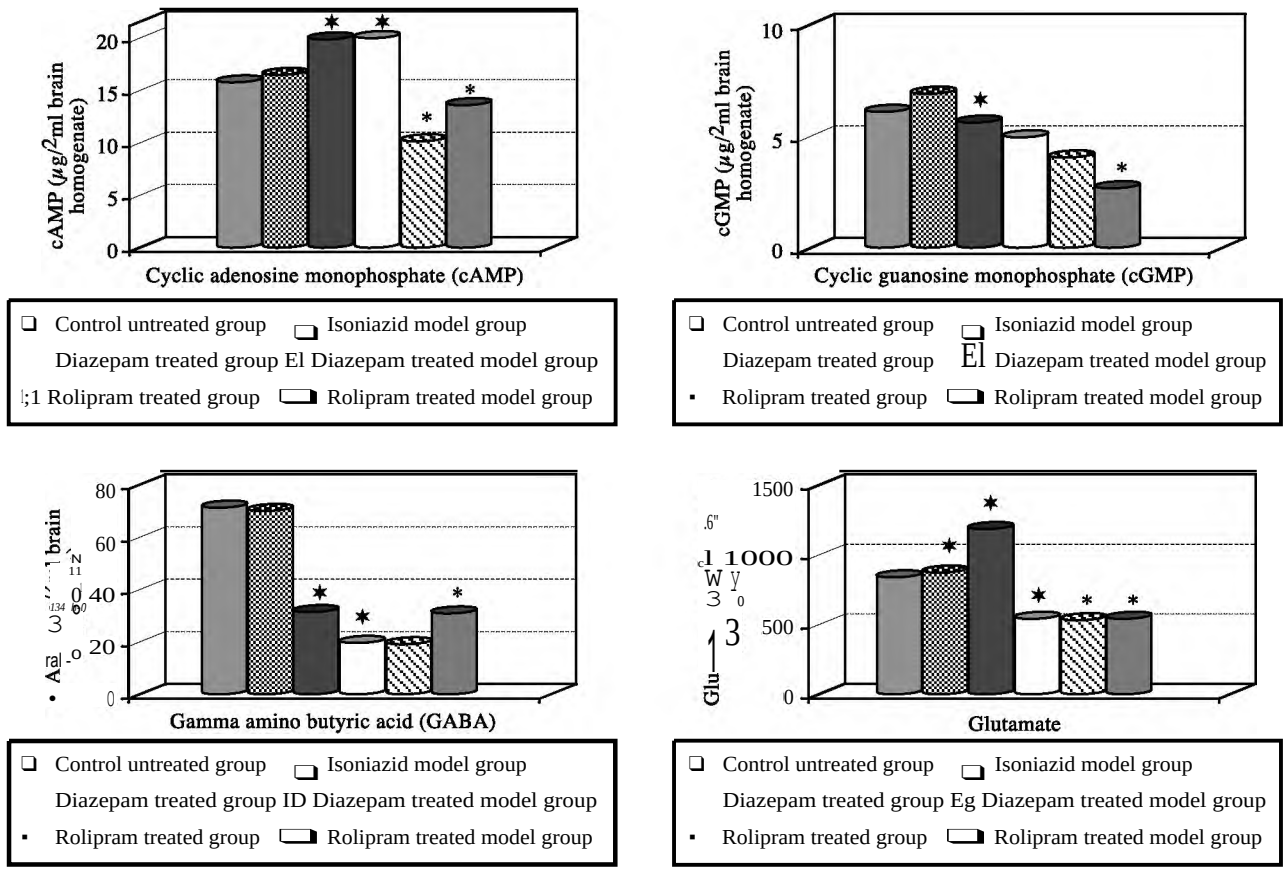


Fig. (2): Effects of rolipram (single i.p. 0.5 mg/kg, 10 min. after i.p. 250 mg/kg INH), compared to diazepam (single i.p. 10 n2g/kg, 10 min. after i.p. 250 mg/kg INH) on brain homogenate biochemical parameters (a) cAMP, (b) cGMP, (c) GABA and (d) Glutamate (i4g/zml brain homogenate) (mean±SD).
* Significant compared to normal rats
* Significant compared to INH seizure model.

Results of pentylenetetrazole kindling model:

Compared with the untreated PTZ kindled rats (group II), a challenge dose of 0.5mg/kg i.p. Rol, ten minutes after i.p. 30mg/kg PTZ, following repeated Rol injections (group IIIB), led to significant reduction in seizure severity, with earlier onset and longer duration. However, this improvement was much less than that of DZ, with earlier onset and almost equal duration (Tables 5,6).

Relative to the kindling model group (II), chronic Rol treatment (group IIIC) reversed the PTZ - induced reductions in ß power, versus rise in δ power. These variations were similar to chronic DZ treatment (group IIIB) as regards reversal of raised δ power (Table 7, Fig. 4).

The changes induced by chronic Rol treatment of kindled rats (group IIIB) in relation to the untreated epileptic rats (group II) were associated with underlying significantly increased brain GABA/glutamate ratio, together with unchanged cAMP/cGMP ratio. These Rol-mediated biochemical changes were similar but less than those induced by repeated DZ injections (Table 8, Fig. 5).

Compared to the control group (group IA), after repeated Rol administration, a challenge dose of 0.5mg/kg Rol (group IC) resulted in worsening of seizure severity, but with delayed onset and shorter duration, in contrast to DZ, which showed similar behavior to normal rats (group IA), with almost equal onset and duration (Tables 5,6).

Relative to normal rats (group IA), Rol treated controls (group IC) were noticed to have similar EEG recordings regarding power of source, α, θ and δ waves, except for ß power, which was significantly reduced. This was less than DZ-induced decrease ß power, while similar to DZ, concerning α and δ waves. But was found to be in contrast to DZ-mediated decreased power of source wave against elevated θ power (Table 7, Fig. 4).

Comparing Rol treated controls (group IC) with normal rats (group IA), biochemical analysis revealed significantly increased brain GABA/ glutamate ratio, accompanied by lower cAMP and unchanged cGMP. These changes were less than DZ treated controls (group IB) for the reductions in GABA and cAMP, but similar to DZ in the unchanged cGMP and opposite to the increased glutamate (Table 8, Fig. 5).

Table (5): Effects of rolipram, compared to diazepam, in control and PTZ kindling model on seizure severity (modified Racine staging from 0 to 6) in awake rats (mean±SD).

Group	Control group (group IA)	Diazepam treated control (group IB) [Repeated i.p. 10mg/kg]	Rolipram treated control (group IC) [Repeated i.p. 0.5 mg/kg]	PTZ kindling model group (group II) [Repeated i.p. 30 mg/kg]	Diazepam-treated kindling model group (group IIIA) [Repeated i.p. 10 mg/kg DZ, 10 minutes after each i.p. 30 mg/kg PTZ]	Rolipram - treated kindling model group (group IIIC) [Repeated i.p. 0.5 mg/kg DZ, 10 minutes after each i.p. 30 mg/kg PTZ]
Modified Racine staging for seizure severity (Mean±SD)	0	0	1.13±0.84*	4.88±0.79*	1.38±0.52✶	2.75±0.46✶♦

* Significant compared to control group.
✶ Significant compared to PTZ kindling model group.
♦ Significant compared to diazepam treated kindling model group.

Table (6): Effects of rolipram, compared to diazepam, in control and PTZ kindling model on latency (min.) and duration (min.) of seizures in awake rats (mean±SD).

Seizures peak	Control group (group IA)	Diazepam treated control (group IB) [Repeated i.p. 10mg/kg]	Rolipram treated control (group IC) [Repeated i.p. 0.5 mg/kg]	PTZ kindling model group (group II) [Repeated i.p. 30 mg/kg]	Diazepam-treated kindling model group (group IIIA) [Repeated i.p. 10 mg/kg DZ, 10 minutes after each i.p. 30 mg/kg PTZ]	Rolipram - treated kindling model group (group IIIC) [Repeated i.p. 0.5 mg/kg DZ, 10 minutes after each i.p. 30 mg/kg PTZ]
Latency	0	0	4.88±5.38	4.94±4.35	14.75±8.08	0.91±0.3
Total Duration	120	120	61.1±62.95	6.75±6.86	1.21±1.04	5.38±3.58

Table (7): Effects of rolipram, compared to diazepam, in control and PTZ kindling model on power spectral density (PSD) [amplitude^2/frequency] (j4V^2/Hz) of source wave, beta, alpha, theta and delta waves in anesthesized rats (chloral hydrate) (meantSD).

Power spectral density (PSD) Mean±SD	Control untreated group	Diazepam treated group (Repeated 10 mg/kg, i.p.)	Rolipram treated group (Repeated 0.5 mg/kg, i.p.)	PTZ kindling model (Repeated 30 mg/kg, i.p.)	Diazepam treated kindling model group (10 mg/kg, i.p., 10 min. after PTZ)	Rolipram treated kindling model group (0.5 mg/kg, i.p., 10 min. after PTZ)
Source Wave	70.54±5	43.1±3.63*	72.49±4.92	45.42±4.23*	29.33±3.09*	78.95±8.38*
Beta Wave	66.55±4.46	16.37±1.31*	26.77±4.1*0	38.18±2.42*	18.14±3.1*	96.52±6.29 *
Alpha Wave	6.03±0.74	8±3.85	8.07±2.21	2.19±1.29*	2.76±0.7	2.81±0.62
Theta Wave	11.93±2.94	22.45±8.21*	18.95±7.35	8.88±1.79	5.03±0.74	4±1
Delta Wave	13.11±2.64	27.71±15.15	11.41±2.23	41.72±7.66*	1.58±0.35*	3±0.98*

* Significant compared to control untreated group. OSignificant compared to diazepam treated group.
* Significant compared to PTZ kindling model group.

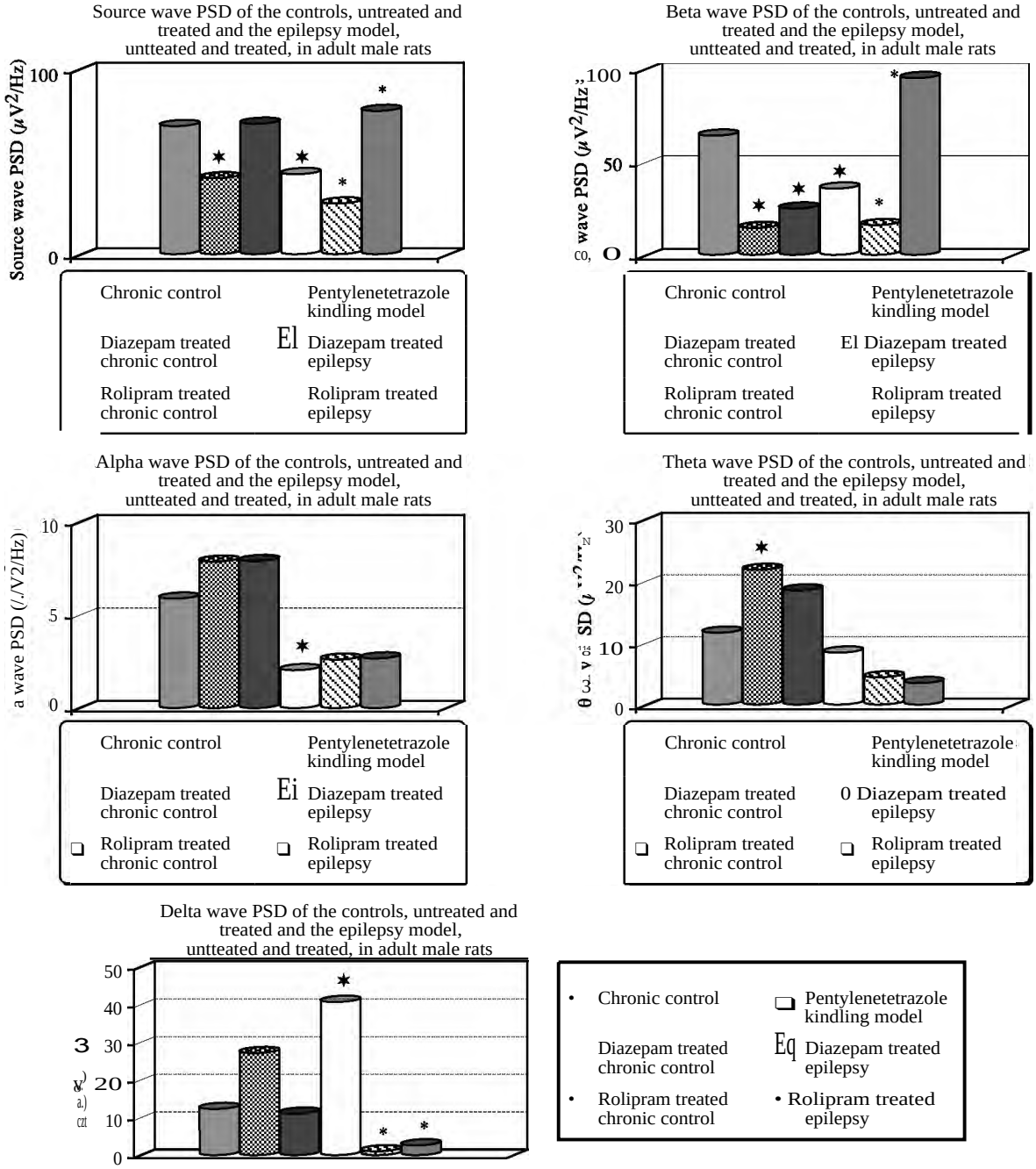


Fig. (3): Effects of rolipram (challenge i.p. 0 5 mg/kg after 5 days abstinence, following repeated dosing once every other day for one month, then once every other 2 days for another month, each dosing 10 min. before i.p. 30 mg/kg PTZ), compared to diazepam (challenge i.p. 10 mg/kg after 5 days abstinence, following repeated dosing once every other day for one month, then once every other 2 days for another month, each dosing 10 min. before i.p. 30 mg/kg PTZ), on PSD of (a) Source wave, (b) Beta wave, (c) Alpha wave, (d) Theta wave and e) delta wave 04V/Hz^2) in anesthesized rat (chloral hydrate 50mgl/kg) (mean±SD).

Table (8): Effects of rolipram, compared to diazepam, in control and PTZ kindling model on brain homogenate biochemical parameters (cAMP, cGMP, GABA and glutamate) (μg/g brain weight) in rats (mean±SD).

Biochemical parameter	Control untreated group	Diazepam treated control group (Repeated 10 mg/kg, i.p.)	Rolipram treated control group (Repeated 0.5 mg/kg, i.p.)	PTZ kindling model group (Repeated 30 mg/kg, i.p.)	Diazepam treated kindling model group (Repeated 10 mg/kg, i.p., 10 min. after PTZ)	Rolipram treated kindling model group (Repeated 0.5 mg/kg, i.p., 10 min. after PTZ)
cAMP	20.79±1.76	6.65±0.69*	13.46±0.33*◊	27.86±1.35*	23.2±1.53✶	23.88±1.94✶
cGMP	8.06±1.44	6.9±0.58	7.47±1.41	17.64±1.26*	15.46±1.58✶	14.81±1.4
cAMP/cGMP	2.64±0.4	1±0.17*	1.87±0.43*◊	1.59±0.19*	1.51±0.15	1.63±0.23
GABA	78.58±1.23	54.82±1.69*	73.55±1.65*◊	124.88±1.68*	113.4±1.95✶	105.36±1.41✶♦
Glutamate	570.54±0.9	1448.93±1.81*	398.21±1.3*	801.33±1.91*	97.44±1.19✶	166.41±1.88✶♦
GABA/glutamate	0.14	0.04*	0.19±0.01*	0.16*	1.16±0.03✶	0.63±0.01✶♦

*Significant compared to control untreated group.
◊Significant compared to diazepam treated control group.
✶Significant compared to PTZ kindling model group.
♦Significant compared to diazepam treated kindling model group.

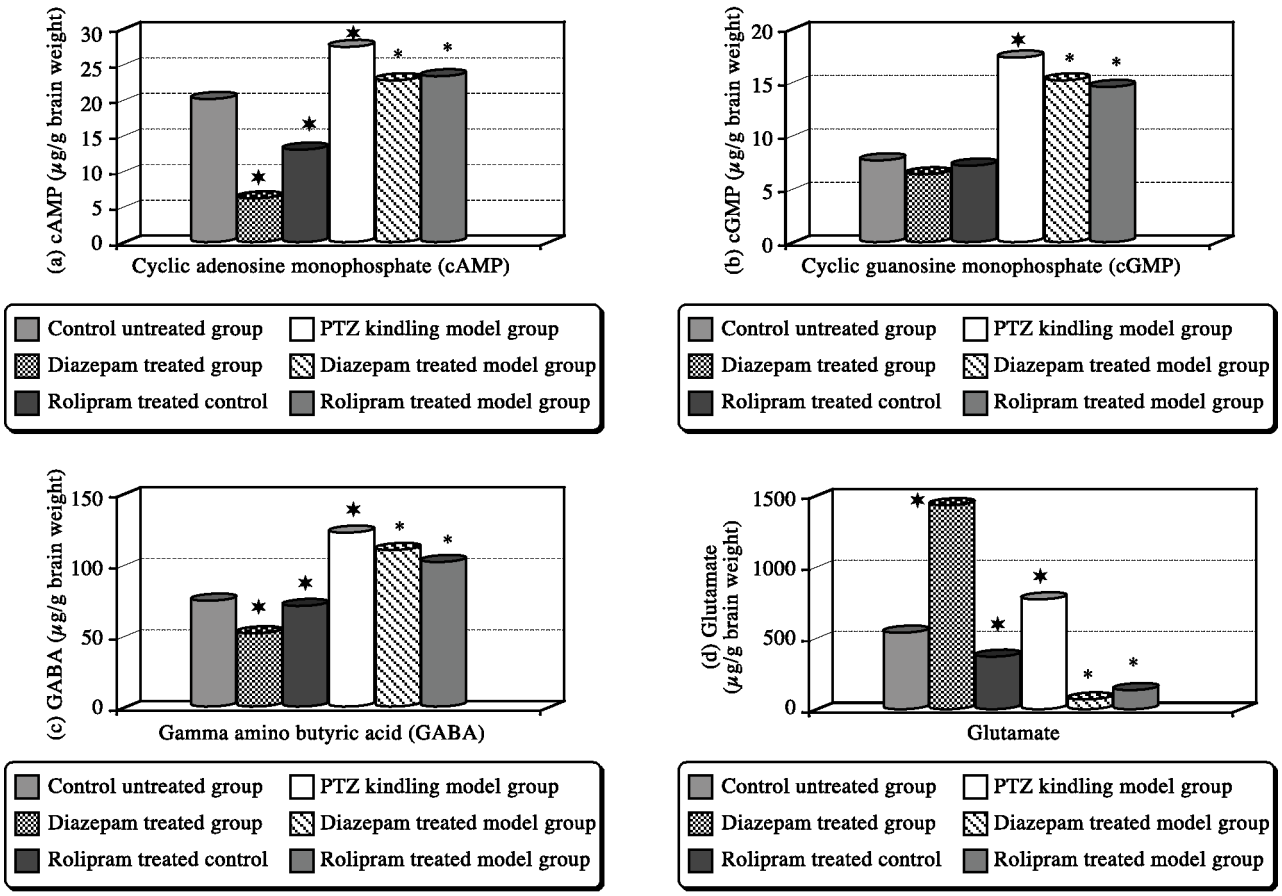


Fig. (4): Effects of rolipram (challenge i.p. 0.5 mg/kg after 5 days abstinence, following repeated dosing once every other day for one month, then once every other 2 days for another month, each dosing, 10 min. after i.p. 30 mg/kg PTZ), compared to diazepam (challenge i.p. 10 mg/kg after 5 days abstinence, following repeated dosing once every other day for one 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 (μg/g brain weight) (mean±SD).
✶Significant compared to normal rats. *Significant compared to INH seizure model.

Discussion

Isoniazid-induced seizures:

As in the current experiment, Rol-induced higher 6 power was suggested to be a negative finding, even with apparently normal clinical state, and is predictive of the re-occurrence of seizures [33].

In agreement with Rol-mediated increased brain glutamate, cAMP or cAMP/cGMP ratio, previous work demonstrated that high dose s.c. rolipram (2.75mg), whether systemic or intracerebroventricular (i.c.v.) increased cAMP in hippocampal homogenate, accompanied by a restoration of chemically-induced NMDA receptors hypofunction [34].

Consistent with Rol-induced decreased cAMP, a small dose of i.p. rolipram (0.3mg/kg) did not succeed to increase cAMP in the work of Giorgi et al. [35]. However, acute i.p. injection of 3mg/kg rolipram was able to increase cAMP, transiently, for up to 24 hours post injection as was also demonstrated by Frey et al. [36].

Luszczki et al. [37] explained that cAMP decrease prevented a cAMP-facilitated hyperpolarization, which, subsequently, attenuated the depolarizing current (Ih) of a voltage-activated Na+/K+ channel and raised maximal electroshock seizure threshold in adult male Swiss mice.

In the present investigation, Rol in healthy rats was found to increase both cAMP and glutamate with the reverse in INH seizure model, relative to normal rats. This was attributed to the ability of Rol to enhance NMDA-mediated increased cAMP in neurons [38], meaning that NMDA receptor stimulation resulted in modified cAMP production via PDE4 [39].

A similar proconvulsant effect of Rol was noticed in a study testing effects of daily i.p. 0.01 and 0.1mg/kg rolipram for 4 weeks in young and aged rats on excitatory neurotransmission, Rol resulted in preferential NMDA receptors binding [40].

As with the present study, the Rol-mediated increase in frontal cortex GABA level was observed upon chronic i.p. injection of 0.1mg/kg rolipram, once daily for 3 weeks, to male albino mice, 20-25g, exposed to chronic mild stress [41].

In the current research, both GABA and glutamate, upon Rol administration, were either increased relative to the INH seizure model or decreased compared to the normal rats. The link between both neurotransmitters was detected by considering that acetate is converted in glial cells to glutamine, the byproduct and precursor of glutamate, to be taken up by GABAergic neurons and converted to GABA [42].

Advantageously, It was found that using a PDE-4 inhibitor analog by oral gavage combined with INH resulted in enhanced antituberculous activity in mice [43] and rabbit pulmonary TB model [44], similar patterns were observed when using PDE-3 and PDE-5 inhibitors [45].

Pentylenetetrazole kindling model:

In the current study, rolipram was noticed to show raised high-frequency wave (ß) with less spindle activity (0), suggestive of reduced seizure severity [46].

In the present study, rolipram mediated a decrease in the slow S wave in kindled rats, taken as a sign of improved cognitive functions related to cerebral blood flow, a state opposite to sleep and anesthesia [47].

As in the present experiment, Shalaby and Kamal [41] found that rolipram resulted in decrease GABA level in mice exposed to chronic mild stress, through cAMP-regulated mechanisms.

As in the present experiment, rolipram caused a decline in cAMP, whether in normal or epileptic rats, this came in accordance to the fact that a rebound decrease in cAMP can follow chronic high doses of rolipram [48], together with secondary modification of glutamatergic receptors through [49].

In conjunction to the possible antiepileptic effect of chronic rolipram through reduced cAMP, an increase in cAMP level was taken as a marker of reduced sensitivity of hyperpolarization, cyclic nucleotide-gated channels (HCN) preceding the beginning of epilepsy [46]. These channels are known to diminish the efficiency of incoming postsynaptic potentials [50].

In accordance to the current rolipram-induced cGMP decrease in epileptic rats, lowering of cGMP, with or without increased cAMP, in PTZ-induced seizures in mice was correlated to reduced seizure severity with delayed onset and prolonged duration, versus earlier onset associating the increased cGMP [51].

It is evident that cGMP have both direct and indirect effect on glutamate neurotransmission. The direct effect is supposed to be biphasic as denoted by a decrease in presynaptic glutamate release from neurons of hippocampus and cerebral cortex upon increased cGMP level, mediated by its analogs or PDE inhibition, with a later NO/cGMP inhibition of glutamate reuptake [52].

The controversy about cGMP effect in convulsions depends on animal species, type of seizures, experimental seizure models, brain regions involved and dose of PDE inhibitor, ranging from neuroprotection with low concentrations to neurotoxicity in high concentrations [53].

Moreover, a rolipram-attenuated neurotoxicity following global ischemia in rats and ischemic stroke in mice [54], might share in its assumed antiepileptic efficacy.

More studies are recommended to clarify the role of rolipram and explore sites and mechanisms of action in epilepsy.

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الملخص العربى

مقدمة : مرض الصرع عبارة عن نوبات تشنجية تلقائية جراء تأثر الأعصاب فى المخ بشحنات كهربائية متزامنة. يعد مرض الصرع من الأمراض العصبية المنتشرة على مستوى العالم، حيث يبلغ اجمالى المصابين به حوالى ١٪ من اجمالى سكان العالم. كما أن الصرع يمثل عبئا اقتصاديا نظرا لتكاليف العلاج من ناحية وما يؤدى اليه المرض من خلل فى التعليم وفرص العمل والاكتئاب المصاحب لعدم القدرة على اقامة علاقات اجتماعية سوية، بالاضافة لبعض الأمراض الملازمة له. عقار الروليبرام يعمل على زيادة السايكليك أدينوزين مونوفوسفيت من خلال تثبيط انزيم فوسفودایاستریز-٤ والذى ويتم تجربته حاليا فى علاج الالزهايمر والسكتة الدماغية والفصام والاكتئاب.

الهدف من الدراسة : محاولة اكتشاف تأثير عقار الروليبرام، مقارنة بالديازيبام، على كل من التشنجات المحدثة بالايونيازيد ومرض الصرع الناتج عن تكرار تعاطى البنتايلينتيترازول.

خطة البحث : تم تقسيم الجرذان الذكور البالغة الى ثلاث مجموعات أساسية: أولا: المجموعات القياسية والتى اما لا تتعاطى أى عقار، أوتتعاطى الديازيبام ١٠مجم/كجم عن طريق الحقن فى الغشاء البريتونى أو يتم حقنها بالروليبرام ،٥ .مجم/كجم عن طريق الحقن فى الغشاء البريتونى، كما فى الديازيبام. ثانيا: مجموعة التشنجات الناتجة عن حقن ٢٥٠ مجم/نجم ايزونيازيد فى الغشاء البريتونى، اما معالجة بالديازيبام أو الروليبرام بعد عشر دقائق من حقن الأيزونيازيد، كما سبق، أو غير معالجة. ثالثا: مجموعة الصرع المحدثة عن طريق التعاطى طويل المدى للبنتيلينتيترازول ٣٠مجم/كجم عن طريق الحقن فى الغشاء البريتونى مرة واحدة كل يومين لمدة شهر ثم مرة كل ٣ أيام لمدة شهر اخر، اما معالجة بالديازيبام أو الروليبرام، بعد عشر دقائق من حقن البنتيلينتيترازول، كما سبق، أو غير معالجة.

يتم ملاحظة الجرذان بعد التعاطى لمدة ساعتين وتصويرها بالفيديو لتحديد الزمن منذ التعاطى وحتى حدوث أول تشنج، المدة الاجمالية للتشنجات وتحديد شدة التشنج على القياس المعدل لراسين أو يتم عمل رسم مخ للجرذان لمدة ساعتين تحت تأثير مخدر الكلورال هيدريت. يتم تشريح المخ ووزنه على ميزان كهربى بالنسبة للمجموعات الثلاث ثم تحويله الى متجانس لقياس السايكليك أدينوزين مونوفوسفيت والسايكليك جوانوزين مونونوفوسفيت والجابا والجلوتاميت وحساب النسبة ما بين السايكليك أدينوزين مونوفوسفيت والسايكليك جوانوزين مونونوفوسفيت والنسبة ما بين الجابا والجلوتاميت.

النتائج : غياب تحسن سلوكى ذى دلالة احصائية فى شدة التشنجات المحدثة باليزونيازيد بعد تعاطى الروليبرام، بعكس الديازيبام. كما ان رسم المخ أظهر تشابه فى قوة الموجة الأساسية والموجتين البطيئتين (ثيتا ودلتا) مع المجموعة التى أحدث الأيزونيازيد بها تشنجات، بينما انخفضت طاقة الموجتين السريعتين (بيتا وألفا)، بعكس الديازيبام فيما يخص الموجة السريعة (بيتا). كمت أدى الروليبرام الى من الزيادة فى الجلوتاميت، مما أدى الى ارتفاع النسبة بين الجابا والجلوتاميت، بعكس الديازيبام، غياب تغير فى النسبة ما بين السيكليك أدينوزين مونوفوسفيت والسيكليك جوانوزين مونوفوسفيت، بعكس الديازيبام.

بينما تحسنت شدة التشنجات بفارق له دلالة احصائية عن مجموعة الصرع المحدثة بالبنتيلينتيترازول الغير معالجة، لكن بصورة أقل من العلاج بالديازيبام. أظهر رسم المخ ارتفاعا ذا دلالة احصائية لقوة الموجةالسريعة (بيتا)، بعكس الديازيبام، بينما انخفضت قوة الموجة البطيئة (دلتا)، مماثلا للديازيبام، بينما لم تتغير الموجتان (ألفا وثيتا)، كما فى الديازيبام. عند تحليل متجانس المخ كيميائيا، تبين ارتفاع النسبة بين الجابا والجلوتاميت، لكن بصورة أقل من الديازيبام، فى غياب تغير للنسبة ما بين السيكليك أدينوزين مونوفوسفيت والسيكليك جوانوزين مونوفوسفيت فى المخ، كما حدث مع الديازيبام.

مما سبق يمكن استخلاص التالى:

أظهر الروليبرام فى الجرذان الذكور البالغة التى تعانى من التشنجات المحدثة بالأيزونيازيد ميلا لاحداث تشنجات، قد يكون مضافا للتأثيرات التشنجية للأيزونيازيد.

من خلال هذه الدراسة، قد يكون هناك محاذير لامكانية استمرارتعاطى الروليبرام فى وجود التشنجات الحادة اناتجة عن التسمم الدوائى.

استطاع الروليبرام أن يكون له تأثيرا فعالا فى التخفيف من الصرع الناتج عن البنتيلينتيترازول فى الجرذان الذكور البالغة، وان كان تأثيره أقل من الديازيبام.

من خلال هذه الدراسة، هناك احتمالات لامكانية استخدام الروليبرام فى علاج مرض الصرع.

من التوصيات اللازمة، تكرار الدراسة على فصائل أخرى، باستخدام طرق أخرى لاحداث التشنجات مع استخدام الروليبرام مضافا الى دواء معروف استخدامه كمضاد للتشنجات فى الدراسات القبل الاكلينيكية.