RESEARCH PAPER

Acta Neurobiol Exp 2026, 86
DOI: 10.55782/XXX

Chronic pinacidil attenuates the pentylenetetrazole-induced kindling in rats

Artur Teixeira Pereira[1,2], Erica Aparecida Gelfuso[1], Luiz Luciano Falconi-Sobrinho[1,2], Rene Oliveira Beleboni[1,2,3]*

[1] Department of Biotechnology, University of Ribeirão Preto, Ribeirão Preto São Paulo, Brazil
[2] Medical School, University of Ribeirão Preto, Ribeirão Preto São Paulo, Brazil
[3] Carlos Chagas Institute – Fiocruz-PR, Curitiba Paraná, Brazil
** Email: rbeleboni@unaerp.br*

Seizures in epilepsy result from excessive neuronal electrical discharges. Imbalances in potassium homeostasis can increase neuronal excitability and trigger epileptic seizures. Drugs that enhance potassium currents are expected to suppress seizures. Pinacidil, an ATP-sensitive potassium channel opener, has shown anticonvulsant effects in acute seizure models in rodents. However, its effects following chronic systemic administration in pentylenetetrazole (PTZ)-kindled rats remain unknown. The aim of this study was to investigate the impact of long-term pinacidil treatment in the PTZ-kindling model of epilepsy. Male Wistar Hannover rats were treated intraperitoneally with pinacidil 30 min before each PTZ injection, administered intraperitoneally every other day for 27 days (14 injections). Behavioral responses were recorded for 30 min immediately after each PTZ injection. Twenty-four hours after the last PTZ injection, animals underwent the rotarod test and were then euthanized for toxicological analysis of renal and hepatic biochemical markers in serum. Chronic pinacidil treatment prevented the development of PTZ-induced kindling. Biochemical data showed that chronic pinacidil did not provoke changes in serum creatinine and urea levels or in aspartate aminotransferase and alanine aminotransferase levels. These findings suggest that long-term administration of pinacidil impairs the progression of PTZ-induced kindling without causing nephrotoxic or hepatotoxic effects.

Key words: anticonvulsant, epilepsy, kindling, pentylenetetrazole, pinacidil, seizures

INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by recurrent seizures caused by abnormal and excessive synchronous firing of a group of brain cells (Engel, 2001; England et al., 2012; WHO, 2019). Changes in potassium conductance have been shown to be involved in the seizure process by increasing or decreasing neuronal excitability (Benarroch, 2009; Wang et al., 2016; Ågren et al., 2019). Extracellular ionic components such as potassium have been shown to be increased during seizure-like events when compared to non-seizure states (Kohling et al., 1995; Somjen, 2002). Indeed, preclinical evidence demonstrates that impairment of potassium conductance by intracerebroventricular injections of voltage-gated potassium channel inhibitors increases neuronal excitability, resulting in seizures (Gandolfo et al., 1989a, b). In contrast, drugs that increase potassium currents have been shown to decrease the frequency of spike waves (Acar et al., 2016) and acute seizures (Del Pozo et al., 1990; Zhao et al., 2023, 2024), as well as the expression of genes related to the pathophysiology of epilepsy (Kiliç & Soytürk, 2022).

In this scenario, the ATP-sensitive potassium channel opener pinacidil has been a target of preclinical studies in epilepsy due to its putative neuroprotective properties (Acar et al., 2016; Duzcu et al., 2022). However, the literature has shown conflicting results

Received 6 August 2025, accepted 23 January 2026

regarding the effects of pinacidil on pharmacologically induced acute seizures. Studies performed by Del Pozo et al. (1990) demonstrated that intracerebroventricular injections of pinacidil failed to reduce pentylenetetrazole (PTZ)-induced seizures exhibited by mice. Conversely, studies performed by Pereira et al. (2024) showed that intraperitoneal injections of pinacidil attenuated PTZ-provoked tonic-clonic seizures in Wistar rats. In line with this latter finding, a study using electrographic recordings from the somatomotor cortical area of epileptic rats showed that intraperitoneal administration of pinacidil was able to decrease the frequency of spike waves of epileptiform activity evoked by intracortical injection of penicillin (Acar et al., 2016).

In addition to the literature presenting divergent results on the effects of pinacidil in acute seizures, little is known about the long-term effects of pinacidil in experimental chronic epilepsy.

Therefore, aiming to investigate the long-term effects of pinacidil on chronic seizures, we evaluated the behavioral responses exhibited by PTZ-kindled rats that were intraperitoneally pretreated with pinacidil (10 mg/kg, 30 min before each PTZ injection). The dose and pretreatment interval were selected based on prior studies demonstrating anticonvulsant efficacy without significant toxicity (Pereira et al., 2024) and pharmacokinetic data indicating peak plasma levels within 30–60 min after intraperitoneal administration in rodents (e.g., rapid absorption and onset of action for ATP-sensitive potassium channel openers). Furthermore, we examined the possible side effects of chronic pinacidil treatment on renal and hepatic marker levels as well as locomotor integrity.

METHODS

Subjects and ethics

Male Wistar rats (*Rattus norvegicus*, *Rodentia, Muridae*) weighing 150-180 g (n=6-8 per group) from the Anilab company (Paulínia, SP, Brazil) were studied. They were housed 4 to a cage (41 × 34 × 17.8 cm), with water and food ad libitum. The enclosure was maintained under a light/dark cycle of 12/12 h (lights on from 6 a.m. to 6 p.m.), and at a relative humidity (55 ± 10%) and standard temperature (22°C ± 2°C). All effort was made to minimize animal suffering. Housing and all experimental protocols adhered to the Guide for the Care and Use of Laboratory Animals ("Guide for the Care and Use of Laboratory" – Eighth Edition; National Research Council, 2014) and were performed in accordance with the recommendations of the Local Ethics Committee in Research at UNAERP (no. 0011/2023) rules and legal approval. This manuscript also follows the statements of the ARRIVE guidelines 2.0 (Percie du Sert et al., 2020).

Drugs

Pinacidil monohydrate (N-Cyano-N′-4-pyridinyl-N″-(1,2,2-trimethylpropyl; CAS: 85371-64-8; Sigma-Aldrich, cat. no. P1546, St. Louis, MO, USA) at a dose of 10 mg/kg (Pereira et al., 2024) was intraperitoneally administrated before the ionophore GABAergic receptor antagonist Pentylenetetrazole (α,β-cyclopentamethylenetetrazole; CAS Number: 54-95-5; Sigma-Aldrich, cat. no. P6500, St. Louis, MO, USA) at a dose of 35 mg/kg (de Oliveira et al., 2024; Dhir, 2012; Ergul-Erkec et al., 2015).

PTZ-induced kindling

The kindling epilepsy induced by repeated administrations of subconvulsant doses of PTZ is a chronic animal model of epilepsy used for elucidating the epileptogenic mechanisms involved in the genesis of seizures and evaluate the effects of new antiepileptic compounds (Mason & Cooper, 1972; Ergul-Erkec et al., 2015). Here, rats were randomly assigned to two groups: a pinacidil (10 mg/kg i.p.)-treated group and a physiological saline (i.p.)-treated group, 30 minutes before each systemic administration of PTZ. For the development of kindling, PTZ was administered intraperitoneally at a subconvulsant dose of 35 mg/kg every other day for 27 days (14 injections). Kindling was confirmed when the control group that receives PTZ exhibited convulsive episodes (myoclonus of the forelimb with or without loss of the righting reflex) in three consecutive administrations of PTZ (Dhir, 2012). In addition, the duration of the convulsive seizures was also recorded. Immediately after each PTZ injection, animals were placed in a circular arena with transparent acrylic walls (60 cm in diameter and 50 cm in height), with 350 lx fluorescent lighting, and their behaviors were recorded for 30 min with a video camera (Handycam, Sony Corporation, Osaki, Shinagawa-ku, Tokyo, Japan). At the end of each test/animal, the apparatus was cleaned with 10% alcohol. The PTZ-induced convulsive reactions were classified according to the Racine scale, modified by Pinel and Rovner (1978), as shown in Fig. 1. After behavioral recording, all the animals were submitted to the rotarod test and then euthanized, and blood was collected for biochemical analyses.

Score	Motor convulsive reactions
0	no seizure (exploratory behavior)
1	facial movements
2	head myoclonus
3	hind paw myoclonus
4	rearing
5	rearing and falling
6	Myoclonus of the head and ears, forelimb clonus, and rearing and falling events
7	Rotations, violent jumps and vocalization
8	All stage 7 behaviors followed by periods of hypertonia

Fig. 1. Scale of severity of convulsive reactions according to Racine's (1972), modified by Pinel and Rovner (1978).

Rotarod test

The rotarod test has been used to evaluate the balance and motor coordination of rats, enabling investigation of any possibly side-effects of drugs on locomotor function integrity (Coimbra et al., 2001; Dunham & Miya, 1957; Falconi-Sobrinho et al., 2017). The rotarod apparatus for rats used in our work (Acceler rotarod Ugo-Basile 7750, Comerio, Varese, Italy) consists of a revolving rod subdivided into four compartments (width 8.7 cm). 24 hours after the end of the kindling (28th), rats were subjected to three consecutive training sessions on the rotating bar for 5 min each. Then, the animals were subjected to the rotarod test, which was conducted at a constant speed of 8 r.p.m. for 60 s. During the test, the latency to fall from the apparatus was recorded for each animal, with a maximum latency of 60 s (Falconi-Sobrinho et al., 2017).

Serum biochemical analysis

The rats were euthanized immediately after the end of the rotarod test (28th day). For biochemical analysis, blood samples were collected by cardiac puncture into tubes without anticoagulant. Then, the serum was separated by centrifugation at 2500 rpm for 15 min and stored at -20°C until use. Serum levels of creatinine, urea, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed using an autoanalyzer (Mindray BS-380) according to standard spectrophotometric methods using a reagent kit according to the manufacturer's instructions (Labtest, Brazil).

Statistical analysis

Data from the convulsive seizures were submitted to the D'Agostino-Pearson normality test. Data were reported as mean ± S.E.M., and were submitted to a two-way repeated measures analysis of variance (two-way RM ANOVA) followed by Bonferroni´s *post hoc* test. Data from the locomotor function and biochemical measurements were submitted to the Shapiro-Wilk normality test. Data were reported as median with interquartile range, represented as scatter dot plot and submitted to Mann–Whitney *U*-test. In all the statistical analyses *P* values < 0.05 were considered to indicate statistical significance. The software used for statistical analysis and graph plotting was GraphPad Prism version 8.0 (San Diego, CA).

RESULTS

Effects of chronic treatment of the pinacidil on PTZ-induced kindling

According to the two-way RM-ANOVA, there were significant effects of treatment ($F_{1,14}$=135.7; p<0.001; $F_{1,14}$=792.8; p<0.001) and days/time ($F_{13.182}$=8.501; p<0.001; $F_{13.182}$=56,55; p<0.001), as well as an interaction between them ($F_{13.182}$=12.16; p<0.001; $F_{13.182}$=56.84; p<0.001) on kindling and the seizures duration. Intraperitoneal injections of a sub-convulsive dose of PTZ every other day preceded by i.p. injections of the vehicle provoked a gradual increase in seizures severity (kindling; p<0.001) and duration (p<0.001). In contrast, chronic treatment with pinacidil suppressed the progression of kindling by reducing both the severity of seizures from the 11th day onwards and their duration from the 5th day onwards (p<0.001, according to Bonferroni´s *post hoc* test), as shown in Fig. 2 and Fig. 3, respectively.

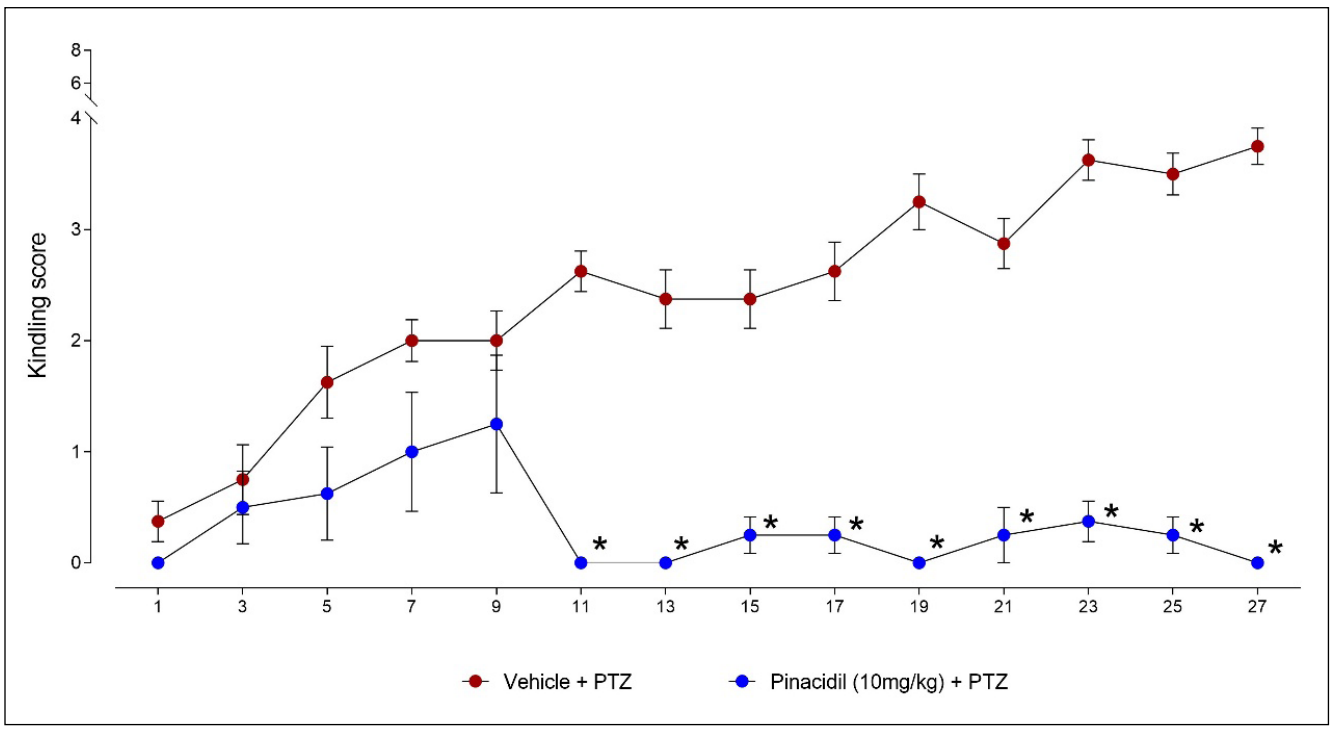

Fig. 2. Effect of chronic administration of either Pinacidil (10 mg/kg) or vehicle (physiological saline) on PTZ-induced kindling. Data are presented as the mean ± standard error of the mean (S.E.M.); * p<0.05 compared with control group (Veh + PTZ at 35 mg/kg i.p.), according to repeated measures two-way ANOVA followed by followed by Bonferroni´s post-hoc test.

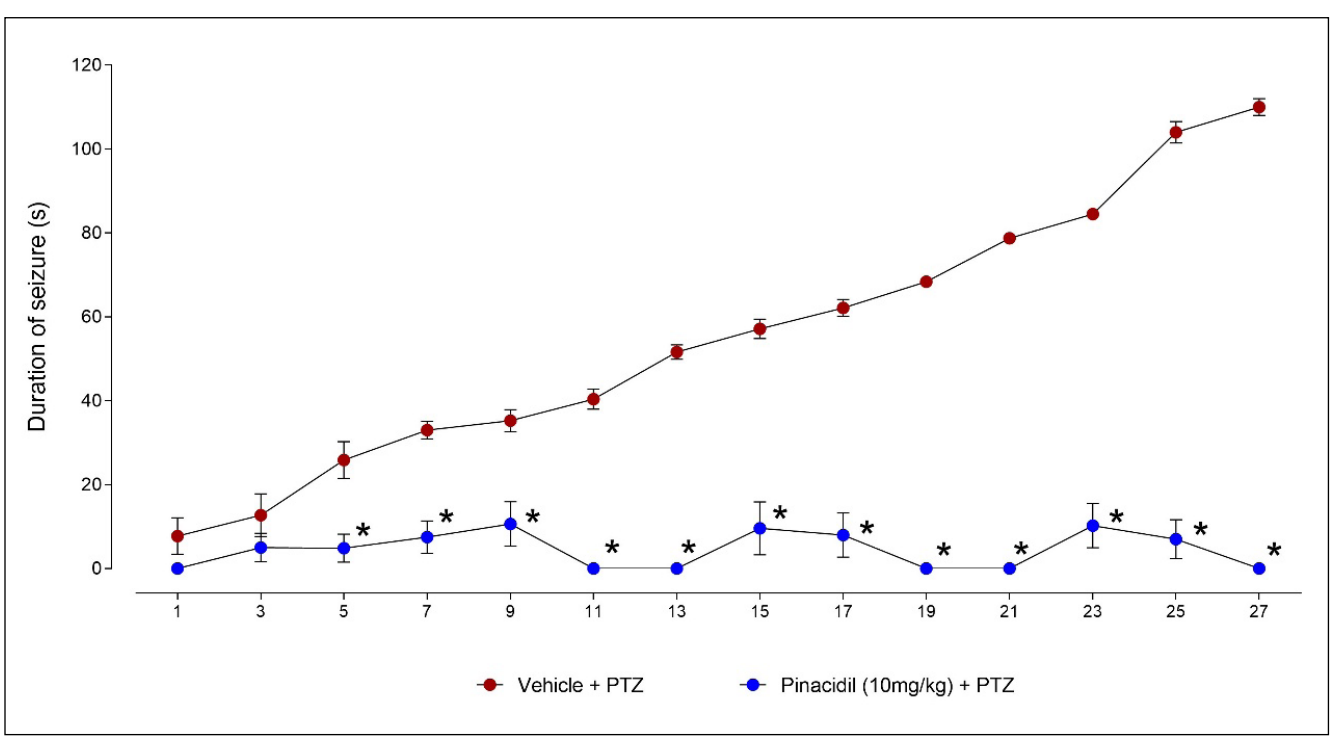

Fig. 3. Effect of chronic administration of either Pinacidil (10 mg/kg) or vehicle (physiological saline) on the duration of seizure in PTZ-induced kindled rats. Data are presented as the mean ± standard error of the mean (S.E.M.); * p<0.05 compared with control group (Veh + PTZ at 35 mg/kg i.p.), according to repeated measures two-way ANOVA followed by followed by Bonferroni´s post-hoc test.

Effects of chronic treatment of pinacidil on locomotor function integrity

According to Mann-Whitney *U*-test, there were no significant effects between the pinacidil and saline treatment on the locomotor functions the PTZ-kindled animals and subjected to the rotarod test (U=28; p=0.999), as shown in Fig. 4.

Effects of chronic treatment with pinacidil on plasma levels in the kidney and liver biomarkers of kindled rats

According to Mann-Whitney *U*-test, there were no significant effects between the pinacidil and saline treatment on levels of creatinine (Mann-Whitney U=15; p=0.338) urea (Mann-Whitney U=10; p=0.1158), AST (Mann-Whitney U=13.5; p=0.254) and ALT (Mann-Whitney U=15; p=0.349) the PTZ-kindled animals, as shown in Fig. 5A-D.

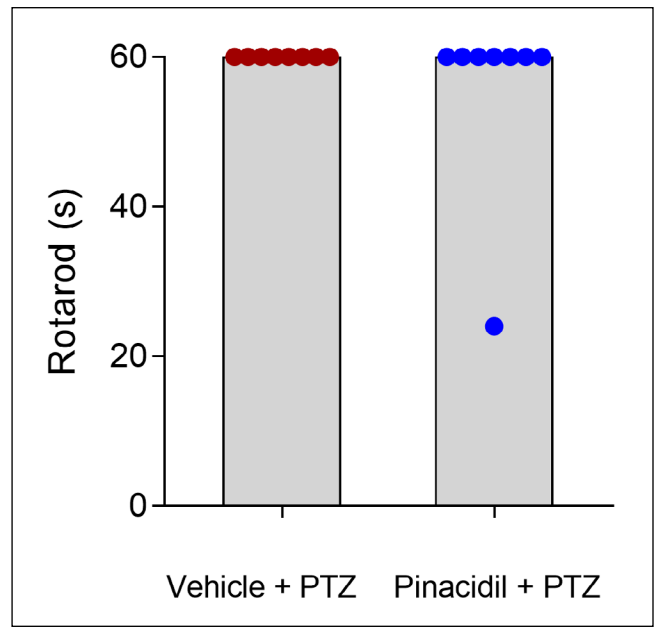

Fig. 4. Lack of effect of chronic administration of either pinacidil (10 mg/kg) or vehicle (physiological saline) on the locomotor integrity, expressed by the latency to fall from the revolving rod during the rotarod test, in PTZ-kindled rats. Data were reported as median with interquartile range and represented as scatter dot plot. $p > 0.05$ compared with control group (Veh + PTZ at 35 mg/kg i.p.), according to Mann–Whitney *U*-test.

DISCUSSION

This study sought to investigate the effects of long-term intraperitoneal administration of pinacidil, an activator of ATP-sensitive potassium channels, against the development of kindling epilepsy induced by injections of the ionophore antagonist to block $GABA_A$ receptor-mediated Cl- influx (PTZ). When PTZ at a dose of 35 mg/kg was preceded by intraperitoneal injections of vehicle, it induced kindling epilepsy, which was characterized by myoclonic movements of the paws and the head, with loss of postural control, but without rotations, violent jumps and vocalization. Our data are in line with previous studies that demonstrated that subconvulsant doses of PTZ over a longer period can cause progressive and permanent convulsive reactions (Ergul-Erkec & Arihan, 2015; Singh et al., 2021; Thapliyal et al., 2023; Pereira et al., 2024). Furthermore, we demonstrated that chronic pretreatment with pinacidil reduced both the severity and duration of seizures displayed by the PTZ-kindled rats.

It is known that abnormal electrical discharges during seizures promote a massive opening of ion channels, including voltage-gated potassium channels, substantially increasing the extracellular concentration of potassium. The accumulation of potassium in the extracellular fluid can reach a threshold level, promoting an increase in neuronal excitability, which in turn increases the extracellular concentration of potassium, establishing a positive feedback mechanism that facilitates the triggering of seizures (Fertziger & Ranck, 1970; Frohlich et al., 2008; Cressman et al., 2009). Furthermore, an increase in potassium also produces depolarizing changes in the reversal potential of the cationic current, which is activated by hyperpolarization, as reported elsewhere (Timofeev et al., 2002). As a result, prolonged activation of cationic channels would result in long-term depolarizations, facilitating neuronal excitability. In fact, the interface between excessive neuronal depolarization and extracellular potassium accumulation is closely linked to the genesis of epilepsy. In turn, pinacidil acts by opening potassium channels in neuronal membranes. The efflux of potassium ions out of the cell makes the inside of the neuron more negative (hyperpolarization), resulting in a reduction in neural excitability (Del Pozo et al., 1990; Acar et al., 2016; Kiliç & Soytürk, 2022; Zhao et al., 2023, 2024). In this context, evidence from studies with genetically engineered rodents has demonstrated that ATP-sensitive potassium channels play a relevant role in preventing triggering and reducing the duration of seizures, mainly through their sulfonylurea receptor 1 (SUR 1) and Kir subunits (Hernández-Sánchez et al., 2001; Yamada et al., 2001; Kiliç & Soytürk, 2022). A study performed by Hernández-Sánchez et al. (2001) found that mice with brain-specific overexpression of SUR 1 were resistant to kainic acid-induced seizures. Con-

currently, Yamada et al. (2001) using knockout mice lacking the Kir subunit, showed that these animals were susceptible to hypoxia-induced generalized seizures. Later, corroborating these latter findings, Kiliç and Soytürk (2022) demonstrated that both the SUR 1 and Kir6.1 subunits of ATP-sensitive potassium channels are involved in the genesis of penicillin-provoked seizures, because their expression levels were

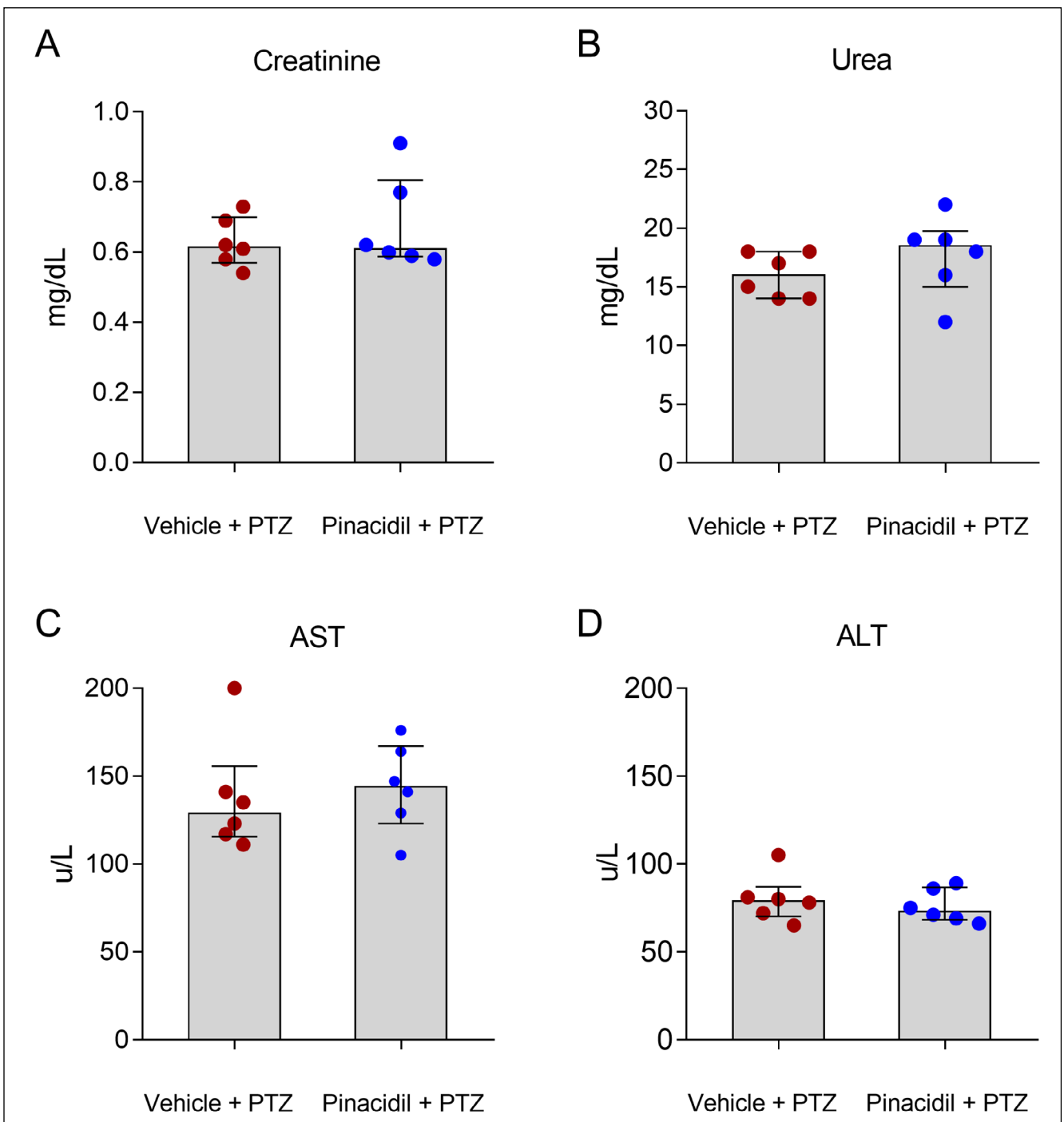

Fig. 5. Lack of effect of chronic administration of either pinacidil (10 mg/kg) vehicle (physiological saline) on renal levels of creatinine (A) and urea (B) and on hepatic levels of aspartate aminotransferase-AST (C) and alanine aminotransferase-ALT (D) in PTZ-kindled rats. Data were reported as median with interquartile range and represented as scatter dot plot. $p>0.05$ compared with control group (Veh + PTZ at 35 mg/kg i.p.), according to Mann–Whitney *U*-test.

increased in both cortex and hippocampus of epileptic rats. Most notably, these latter authors demonstrated that the expression levels of the SUR1 and Kir6.1 gene in epileptic animals were considerably lower when these animals received pinacidil treatment. Considering that Kir6.1 and SUR1 expression is increased in response to seizures, it would be expected that the decrease in seizure activity by pinacidil would reduce the expression of these ATP-sensitive potassium channel subunits.

Electrophysiological findings by Acar et al. (2016) had already demonstrated that pinacidil reduces epileptiform activity by decreasing the frequency of spike waves in a penicillin-induced epilepsy model. In turn, our team recently demonstrated an anticonvulsant action of pinacidil, however, against acute seizures provoked by a higher dose of the GABA receptor antagonist PTZ, as pinacidil was able to increase seizure latencies, while decreasing their severity (Pereira et al., 2024). Indeed, collectively, these studies demonstrate a relevant effect of pinacidil in different models of chemically induced epileptic seizures, highlighting in some cases the involvement of specific subunits of ATP-sensitive potassium channels.

The fact that an ATP-sensitive potassium channel opener, commonly used as a long-term cardioprotective drug, can also provide neuroprotective effects against chronic PTZ-induced seizures has been documented elsewhere (Zhao et al., 2023). As a result, these authors demonstrated that nicorandil, an ATP-sensitive potassium channel opening agent similar to pinacidil, reversed the increase in firing frequency and decreased the magnitude of spikes caused by chronic intraperitoneal injections of PTZ, suggesting that nicorandil can open ATP-sensitive potassium channels of CA1 pyramidal neurons and reduce neuronal excitability, thereby protecting neurons.

Although increasing evidence demonstrates the neuroprotective effects of pinacidil and other ATP-sensitive potassium channel opener similar to it, such as nicorandil, in animal models of epilepsy, to our knowledge, the present study demonstrates the first evidence of the anticonvulsant effects of pinacidil in a chronic animal model of PTZ-induced epilepsy. Intriguingly, the fact that pinacidil was administered 30 min before each PTZ injection, raising the possibility that the observed reduction in seizure severity reflects acute anticonvulsant effects rather than a true disease-modifying (anti-kindling) action. Future studies could address this issue by incorporating either PTZ without the use of drugs after a wash-out period, interim probe sessions without pretreatment and treatment arms initiated after kindling establishment, or by using other animal models that exhibit chronic spontaneous epileptic seizures (e.g., pilocarpine or kainate). Additionally, the lack of EEG recordings precludes detailed analysis of electrographic epileptiform activity.

In this work we also evaluated the hepatic and renal function of PTZ-kindled rats that were pretreated with pinacidil. Our biochemical data showed that systemic administration of pinacidil on alternate days for 27 days did not cause significant changes in AST, ALT, creatinine and urea levels. Furthermore, pinacidil also did not cause locomotor impairment in animals subjected to the rotarod. These data indicate that long-term intraperitoneal administration of pinacidil produced neither nephrotoxic, hepatotoxic effects, nor motor impairment in PTZ-kindled rats. The absence of toxic effects of pinacidil on the liver and kidneys, as well as on motor integrity, was also recently reported by our team in a study that analyzed the protective effects of single administration of this agent against acute minimal and generalized tonic-clonic seizures induced by high doses of PTZ in rats.

In summary, our findings provide novel evidence of the anticonvulsant effects of chronic pinacidil administration in a PTZ-kindling model of epilepsy. Long-term pinacidil treatment reduces both the severity and duration of seizures without inducing motor impairment or renal/hepatic toxicity in kindled rats.

ACKNOWLEDGMENTS

This work was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES) and the Brazilian National Council for Scientific and Technological Development (CNPq).

REFERENCES

Acar, Y., Özmerdivenli, R., Demir, Ş., Beyazçiçek, E., Ankaralı, S., Beyazçiçek, Ö., & Ankaralı, H. (2016). Agonist and Antagonist Effects of ATP-Dependent Potassium Channel on Penicillin Induced Epilepsy in Rats. *Kafkas Journal of Medical Sciences*, *6*(1), 38–45. https://izlik.org/JA72AD25CB

Ågren, R., Nilsson, J., & Århem, P. (2019). Closed and open state dependent block of potassium channels cause opposing effects on excitability – a computational approach. *Scientific reports*, *9*(1), 8175. https://doi.org/10.1038/s41598-019-44564-x

Benarroch E. E. (2009). Potassium channels: brief overview and implications in epilepsy. *Neurology*, *72*(7), 664–669. https://doi.org/10.1212/01.wnl.0000343739.72081.4e

Cressman, J. R., Jr, Ullah, G., Ziburkus, J., Schiff, S. J., & Barreto, E. (2009). The influence of sodium and potassium dynamics on excitability, seizures, and the stability of persistent states: I. Single neuron dynamics. *Journal of computational neuroscience*, *26*(2), 159–170. https://doi.org/10.1007/s10827-008-0132-4

Coimbra, N. C., Freitas, R. L., Savoldi, M., Castro-Souza, C., Segato, E. N., Kishi, R., Weltson, A., & Resende, G. C. (2001). Opioid neurotrans-

mission in the post-ictal analgesia: involvement of mu(1)-opioid receptor. *Brain research*, *903*(1–2), 216–221. https://doi.org/10.1016/s0006-8993(01)02366-6

De Oliveira, D. D., Prinholato da Silva, C., Falconi-Sobrinho, L. L., & Oliveira Beleboni, R. (2024). Longterm effects of vitexin against development of pentylenetetrazole-induced kindling in rats. *Acta neurobiologiae experimentalis*, *84*(3), 266–274. https://doi.org/10.55782/ane-2024-2575

Del Pozo, E., Barrios, M., & Baeyens, J. M. (1990). Effects of potassium channel openers on pentylenetetrazole-induced seizures in mice. *Pharmacology & toxicology*, *67*(2), 182–184. https://doi.org/10.1111/j.1600-0773.1990.tb00808.x

Dhir A. (2012). Pentylenetetrazol (PTZ) kindling model of epilepsy. *Current protocols in neuroscience*, *Chapter 9*, Unit9.37. https://doi.org/10.1002/0471142301.ns0937s58

Dunham, N. W., & Miya, T. S. (1957). A note on a simple apparatus for detecting neurological deficit in rats and mice. *Journal of the American Pharmaceutical Association. American Pharmaceutical Association*, *46*(3), 208–209. https://doi.org/10.1002/jps.3030460322

Duzcu, S. E., Cetinkaya, A., Soyturk, H., & Demir, S. (2021). Effects of the ATP-dependent K (+)-channel effectors pinacidil and glibenclamide on liver tissue in an experimental model of epilepsy: A histopathological study. *Experimental Biomedical Research*, *5*(1), 1–9. https://doi.org/10.30714/j-ebr.2022173846

Engel J., Jr (2001). Classification of epileptic disorders. *Epilepsia*, *42*(3), 316. https://doi.org/10.1046/j.1528-1157.2001.t01-1-36500.x

England, M. J., Liverman, C. T., Schultz, A. M., and Strawbridge, L. M (2012). Epilepsy across the spectrum: Promoting health and understanding. Epilepsy & Behavior, 25 (2), 266–276. https://doi.org/10.1016/j.yebeh.2012.06.016

Ergul-Erkec, O., Arihan, O (2015). Pentylenetetrazole kindling epilepsy model. Turkish Epilepsy Society, 21(1), 6–12.

Fertziger, A. P., & Ranck, J. B., Jr (1970). Potassium accumulation in interstitial space during epileptiform seizures. *Experimental neurology*, *26*(3), 571–585. https://doi.org/10.1016/0014-4886(70)90150-0

Falconi-Sobrinho, L. L., Dos Anjos-Garcia, T., Elias-Filho, D. H., & Coimbra, N. C. (2017). Unravelling cortico-hypothalamic pathways regulating unconditioned fear-induced antinociception and defensive behaviours. *Neuropharmacology*, *113*(Pt A), 367–385. https://doi.org/10.1016/j.neuropharm.2016.10.001

Fröhlich, F., Bazhenov, M., Iragui-Madoz, V., & Sejnowski, T. J. (2008). Potassium dynamics in the epileptic cortex: new insights on an old topic. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry*, *14*(5), 422–433. https://doi.org/10.1177/1073858408317955

Gandolfo, G., Gottesmann, C., Bidard, J. N., & Lazdunski, M. (1989a). Subtypes of K+ channels differentiated by the effect of K+ channel openers upon K+ channel blocker-induced seizures. *Brain research*, *495*(1), 189–192. https://doi.org/10.1016/0006-8993(89)91236-5

Gandolfo, G., Gottesmann, C., Bidard, J. N., & Lazdunski, M. (1989b). Ca2+ channel blockers prevent seizures induced by a class of K+ channel inhibitors. *European journal of pharmacology*, *160*(1), 173–177. https://doi.org/10.1016/0014-2999(89)90669-9

Hernández-Sánchez, C., Basile, A. S., Fedorova, I., Arima, H., Stannard, B., Fernandez, A. M., Ito, Y., & LeRoith, D. (2001). Mice transgenically overexpressing sulfonylurea receptor 1 in forebrain resist seizure induction and excitotoxic neuron death. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(6), 3549–3554. https://doi.org/10.1073/pnas.051012898

Kiliç, U., Soytürk, H. (2022). Investigation of the effects of pinacidil and glibenclamide administration on HCN1, KCNT1, Kir 6.1, SUR1 gene expressions in hippocampus and cortex regions in epileptic rats. *J Exp Clin Med*. 39(3): 868–873.

Köhling, R., Lücke, A., Nagao, T., Speckmann, E. J., & Avoli, M. (1995). Extracellular potassium elevations in the hippocampus of rats with long-term pilocarpine seizures. *Neuroscience letters*, *201*(1), 87–91. https://doi.org/10.1016/0304-3940(95)12136-r

Mason, C. R., & Cooper, R. M. (1972). A permanent change in convulsive threshold in normal and brain-damaged rats with repeated small doses of pentylenetetrazol. *Epilepsia*, *13*(5), 663–674. https://doi.org/10.1111/j.1528-1157.1972.tb04401.x

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D. W., Karp, N. A., Lazic, S. E., Lidster, K., MacCallum, C. J., Macleod, M., Pearl, E. J., … Würbel, H. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS biology*, *18*(7), e3000410. https://doi.org/10.1371/journal.pbio.3000410

Pereira, A. T., Gelfuso, É. A., & Beleboni, R. O. (2024). Repositioning pinacidil and its anticonvulsant and anxiolytic properties in murine models. *Scientific reports*, *14*(1), 22695. https://doi.org/10.1038/s41598-024-73720-1

Pinel, J. P., & Rovner, L. I. (1978). Electrode placement and kindling-induced experimental epilepsy. *Experimental neurology*, *58*(2), 335–346. https://doi.org/10.1016/0014-4886(78)90145-0

Singh, T., Mishra, A., & Goel, R. K. (2021). PTZ kindling model for epileptogenesis, refractory epilepsy, and associated comorbidities: relevance and reliability. *Metabolic brain disease*, *36*(7), 1573–1590. https://doi.org/10.1007/s11011-021-00823-3

Somjen, G., G. (2002). Ion regulation in the brain: implications for pathophysiology. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry*, *8*(3), 254–267. https://doi.org/10.1177/1073858402008003011

Thapliyal, S., Garg, N., Joshi, R., Chakrabarti, A., & Medhi, B. (2023). Pentylenetetrazole Induced Kindling Model of Refractory Epilepsy: A Proof-of-concept Study to Explore Dose and Time Range of Phenobarbital in Rats. *Basic and clinical neuroscience*, *14*(5), 701–712. https://doi.org/10.32598/bcn.2022.3904.1

Timofeev, I., Bazhenov, M., Sejnowski, T., & Steriade, M. (2002). Cortical hyperpolarization-activated depolarizing current takes part in the generation of focal paroxysmal activities. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(14), 9533–9537. https://doi.org/10.1073/pnas.132259899

Wang, L., Dufour, S., Valiante, T. A., & Carlen, P. L. (2016). Extracellular Potassium and Seizures: Excitation, Inhibition and the Role of Ih. *International journal of neural systems*, *26*(8), 1650044. https://doi.org/10.1142/S0129065716500441

World Health Organization (2019). Epilepsy key facts. Available at: https://www.who.int/news-room/fact-sheets/detail/epilepsy (Accessed October 04, 2019).

Yamada, K., Ji, J. J., Yuan, H., Miki, T., Sato, S., Horimoto, N., Shimizu, T., Seino, S., & Inagaki, N. (2001). Protective role of ATP-sensitive potassium channels in hypoxia-induced generalized seizure. *Science (New York, N.Y.)*, *292*(5521), 1543–1546. https://doi.org/10.1126/science.1059829

Zhao, J., Liang, D., Xie, T., Qiang, J., Sun, Q., Yang, L., & Wang, W. (2023). Nicorandil Exerts Anticonvulsant Effects in Pentylenetetrazol-Induced Seizures and Maximal-Electroshock-Induced Seizures by Downregulating Excitability in Hippocampal Pyramidal Neurons. *Neurochemical research*, *48*(9), 2701–2713. https://doi.org/10.1007/s11064-023-03932-w

Zhao, T., Wang, L., & Chen, F. (2024). Potassium channel-related epilepsy: Pathogenesis and clinical features. *Epilepsia open*, *9*(3), 891–905. https://doi.org/10.1002/epi4.12934