Arch Neurosci. 2018 January; 5(1):e64773. doi: 10.5812/archneurosci.64773.

Published online 2017 December 13. **Research Article**

Cromakalim, a Potassium Channel Opener, Ameliorates Organophosphate- and Carbamate-Induced Seizures in Mice

Abbas Norouzi-Javidan,[1] Sattar Ostadhadi,[1,2,3] Samira Zolfaghari,[4] Abouzar Moradi,[5] and Ahmad-Reza Dehpour[2,3,*]

[1]Brain and Spinal Cord Injury Research Center, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[3]Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran
[4]Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
[5]Department of Periodontics, Faculty of Dentistry, Alborz University of Medical Sciences, Karaj, Iran

[*]*Corresponding author*: Ahmad-Reza Dehpour, Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. Tel: +98-2166402569, Fax: +98-2166402569, E-mail: dehpour@yahoo.com

Received 2017 April 17; **Accepted** 2017 November 29.

Abstract

Background: Organophosphates (OPs) and carbamates are acetylcholine esterase inhibitors (AChEIs), which can cause seizure and death. The anticonvulsant properties of potassium channel openers, including cromakalim, have been determined in previous studies.

Methods: In the present experiment, the possible effects of cromakalim on convulsion and death, induced by OPs and carbamates, were studied in mice. Dichlorvos as an OP compound (50 mg/kg) and physostigmine as a carbamate (2 mg/kg) were used to induce seizure in animals.

Results: Cromakalim was injected at doses of 0.1, 10, and 30 μg/kg 30 minutes before dichlorvos and physostigmine administration and 5 minutes before glibenclamide (a potassium channel blocker, 1 mg/kg) administration. All injections were performed intraperitoneally. Following that, the onset of convulsion, death, severity of seizure, and rate of mortality were investigated. The results showed that both dichlorvos and physostigmine induced seizure activity and death in 100% of the animals. Cromakalim at doses of 0.1, 10, and 30 μg/kg significantly increased the latency of both seizure and death ($P < 0.05$). In addition, cromakalim decreased mortality induced by dichlorvos and physostigmine ($P < 0.05$). On the other hand, glibenclamide blocked all the anticonvulsant effects of cromakalim ($P < 0.05$).

Conclusions: This study, for the first time, revealed that cromakalim (an ATP-sensitive potassium channel opener) decreases the rates of seizure and death induced by dichlorvos and physostigmine in mice and presents a new opportunity to manage patients with OP- or carabamate-induced seizures.

Keywords: Organophosphates, Carbamates, K^+ Channel Opener, Seizure, Mice

1. Background

Organophosphates (OPs) and carbamates were primarily used as pesticides in agriculture (1). Some types of OPs have shown therapeutic properties and have been used for the treatment of some neurologic disorders, including Alzheimer disease (2). Both agricultural and medical applications of OPs and carbamates are attributed to their capacity to disrupt the function of the cholinergic nervous system by inhibiting acetylcholinesterase (AChE) (3). This enzyme is responsible for the hydrolysis of acetylcholine (ACh) under physiological conditions; therefore, its blockade leads to ACh accumulation in the nervous system (2).

In humans, poisoning with OPs/carbamates may result in convulsions (4). The cellular mechanisms through which these agents induce seizure are not fully understood (5). Consequently, there is no efficient treatment to manage patients with OP or carbamate-induced seizures. Although previous studies, by relying on the induced disruption of the cholinergic system, have suggested the use of atropine, oximes, and benzodiazepines to control this type of seizure, all these treatments are insufficient and have severe adverse effects (6, 7). Therefore, the mechanisms of seizure induction by OPs and carbamates should be explained, and the importance of studies to discover new therapies and drugs with favorable pharmacological properties and side effect profiles is unquestionable.

Potassium (K^+) channels may play an important role in the control of all features of neuronal excitability, including resting membrane potential (8). The opening of

K^+ channels is expected to hyperpolarize neurons, inhibit action-potential firing, and prevent hypersynchronous neuronal discharge during a seizure (9, 10). K^+ channel opening is one of the potential antiepileptic mechanisms (11, 12). On the other hand, previous reports have shown that adenosine triphosphate (ATP)-sensitive K^+ channels (K_{ATP}), one of different types of K^+ channel family, play an important role in the management of seizure threshold in several in vitro and in vivo models (13, 14). These findings have been confirmed by other clinical trials (15). However, no study has shown the protective effects of K^+ channel openers on seizure induced by OPs and carbamates.

This study was performed to investigate the possible effects of cromakalim (a K^+ channel opener) on OP- and carbamate-induced seizures in mice. We aimed to present possible anticonvulsant agents with specific targets to manage this type of seizure. Therefore, dichlorvos and physostigmine sulfate were used as an OP and carbamate, respectively to induce seizure in mice. Furthermore, the possible effects of K channel openers on convulsion were examined using cromakalim.

2. Methods

2.1. Animals

In total, 120 male NMRI mice, weighing 20 - 26 g, were used in the experiments. The animals were housed in a room with controlled temperature (21 - 22°C) in a 12-hour light-dark cycle at humidity of 50 ± 10%. The animals were allowed free access to standard laboratory food and water. Assignment of subjects to the experimental groups (n, 10 per group) was randomized. The tests were performed between 9 am and 1 pm. The experimental procedures throughout the study were in compliance with the guidelines for the care and use of animals and were approved by the local ethics committee for animal experiments of Tehran University, Tehran, Iran.

2.2. Drugs

The following drugs were used in the experiments: dichlorvos (50 mg/kg) as an OP; physostigmine sulfate (2 mg/kg) as a carbamate; cromakalim (0.1, 10, and 30 μg/kg) as a K channel opener; and glibenclamide (1 mg/kg) as a K channel blocker. As previously reported, the dose of glibenclamide (1 mg/kg) and its solvent (DMSO), administered by the same route, did not significantly change the plasma glucose level (9).

Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate) was obtained from the institute of organic chemistry (Waster green, Iran). Physostigmine sulfate, cromakalim, and glibenclamide were purchased from sigma chemical company. Dichlorvos was mixed with acacia powder and distilled water in a dry mortar at a ratio of 2:1:1.5 (weight/weight/ volume), as previously described (16). Afterwards, saline was added to obtain the desired concentrations. Glibenclamide was dissolved in DMSO, while physostigmine and cromakalim were dissolved in saline. The doses of all drugs were based on the weight of their salt forms.

2.3. Experimental Design

Dichlorvos and physostigmine were administrated intraperitoneally to animals to induce seizures. The 2 experimental groups (dichlorvos and physostigmine-treated) were divided into 3 classes. To investigate the possible role of K channels in OP- and carbamate-induced seizures, we used cromakalim and glibenclamide:

a, Cromakalim (0.1, 10, and 30 μg/kg) was administrated intraperitoneally 30 minutes before carbamate/OP administration.

b, Glibenclamide (1 mg/kg) was administrated intraperitoneally 30 minutes before carbamate/OP injec-

c, To determine the effect of cromakalim on seizure induced by OP/carbamate, 30 μg/kg of cromakalim (effective dose) was administrated 5 minutes before glibenclamide (10).

2.4. Behavioral Assessments

The animals were monitored for 120 minutes after drug injections. Seizures were evaluated and scored based on the staging system by McLean et al. (5, 17): stage 0, no abnormal behavior; stage 1, some abnormal behaviors including salivation, chewing, and pawing at the mouth; stage 2, dazed appearance, intermittent motionlessness, tremor, and/or bobbing of the head; stage 3, random and/or generalized jerks, similar to stage 2; stage 4, intermittent rearing on hind legs with forepaws extended without falling; stage 5, falling to the side or rear, same as stage 4; and stage 6, status epilepticus.

Stages 1 - 3 and 4 - 6 were considered as subconvulsive and convulsive behaviors, respectively. The latency of seizures after drug injection (in seconds), latency until the onset of death within 1 hour (in seconds), mortality after injection within 1 hour (in percentage), and stages of seizures induced by drug injection (in percentage) were recorded.

2.5. Data Analysis

Comparisons between the experimental and control groups were performed by one-way ANOVA, followed by Tukey's post hoc test when appropriate. P value < 0.05 was considered to be significant.

3. Results

3.1. Dichlorvos and Physostigmine Administration Causes Convulsion and Death in Mice

Both dichlorvos (50 mg/kg, ip) and physostigmine (2 mg/kg, ip) triggered seizure activity in mice (Table 1). In these groups, convulsion and death occurred in 100% of the animals (Table 1).

3.2. Effect of Cromakalim on the Onset of Seizure/Death Induced by Dichlorvos and Physostigmine

Pretreatment with cromakalim at doses of 10 μ/kg ($P < 0.05$ for physostigmine; $P < 0.01$ for divhlorvos) and 30 μ/kg ($P < 0.01$ for divhlorvos and physostigmine) increased the latency of clonic seizure (Figure 1) and prevented dichlorvos-induced seizures in mice (Table 1). Also, cromakalim at doses of 0.1 μg/kg ($P < 0.01$), 10 μg/kg ($P < 0.001$), and 30 μg/kg ($P < 0.001$) significantly increased the onset of death after both OP and carbamate administration (Figure 2).

3.3. Effects of Cromakalim on Death Induced by Dichlorvos and Physostigmine

Figure 3 shows that cromakalim administration reduced the percentage of mortality induced by dichlorvos and physostigmine. Cromakalim at doses of 0.1, 10, and 30 μg/kg decreased the mortality percentage, compared to the control group, which received dichlorvos and physostigmine.

3.4. Effects of Cromakalim on Seizure Stages After Dichlorvos and Physostigmine Administration

As presented in Table 1, cromakalim at doses of 0.1, 10, and 30 μg/kg disrupted the convulsive effects of both dichlorvos and physostigmine in mice. This anticonvulsant effect of the K channel blocker showed a dose-dependent manner by increasing the dose of the drug, the percentage of stage 0 and stages 1 - 3 increased, compared to the control group (Table 1).

3.5. Effect of Glibenclamide on the Anticonvulsant Properties of Cromakalim

Administration of glibenclamide (1 mg/kg, ip) 5 minutes before cromakalim injection (30 μg/kg) inhibited the anticonvulsant effect of cromakalim on OP- and carbamate-induced seizures (Table 1) and decreased the latency of clonic seizure ($P < 0.001$) (Figure 1) and time of death after injection ($P < 0.001$) (Figure 2); it also increased the mortality rate (Figure 3).

4. Discussion

In the present study, dicholorvos and physostigmine caused colonic and tonic seizures, followed by death in 100% of animals. After the intraperitoneal injection of dichlorvos or physostigmine, some degree of tremor, hyperactivity, appeared, and the symptoms became more severe, causing death over time. Overall, seizure is one of the adverse effects of poisoning with OPs and carbamates, such as dichlorvos and physostigmine (18, 19). These substances exert their effects by inhibiting AChE in the nervous system.

Depending on the level of AChE inhibition, cholinergic activation may cause hyperactivity of excitable tissues, fasciculation, seizure, convulsion, coma, and death (20). It has been suggested that systemic application of sublethal doses of AChE inhibitors (AChEI) may result in seizures, convulsions, and central nervous system lesions (21). These finding are in agreement with our results, which showed that dichlorvos at a dose of 50 mg/kg and physostigmine at a dose of 2 mg/kg caused convulsion and death in all animals.

Considering the disruption of cholinergic system by the mentioned chemicals, previous studies have suggested atropine, oximes, and benzodiazepines to control this type of seizure. However, drugs typically used against epilepsy in hospitals are ineffective against OP/carbamate intoxication (6). For instance, atropine only alleviates a few symptoms and shows severe adverse effects (22). Moreover, oximes permeate poorly through the blood-brain barrier (23) and cannot affect the CNS. Benzodiazepines are also likely to depress the brainstem respiratory and circulatory centers (7). Therefore, importance of further research to discover new antiepileptic drugs, which can inhibit seizures induced by OPs and carbamates with favorable pharmacological properties and side effect profiles, is unquestionable.

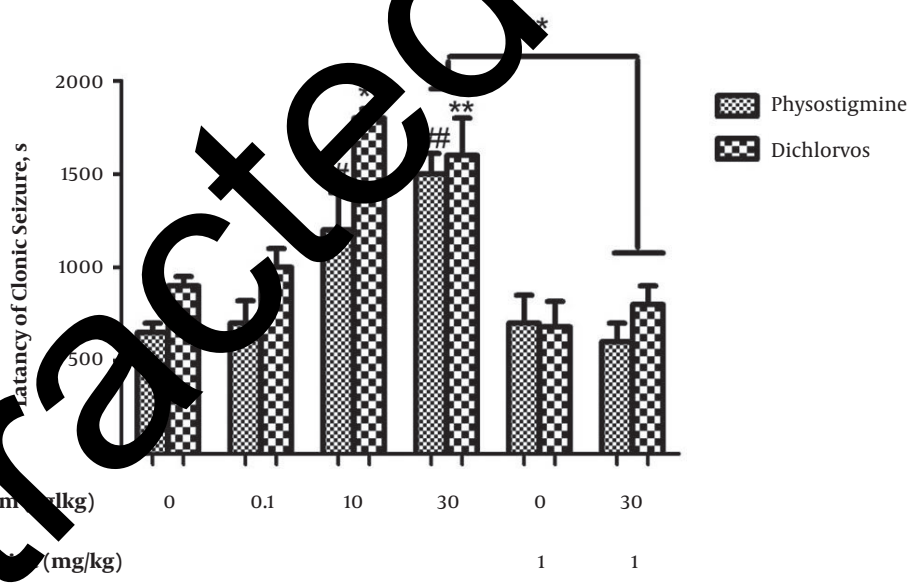
In the present study, cromakalim, a K channel opener, reduced the rates of both seizure and mortality. The onset of seizure and death after OP/carbamate administration reduced in cromakalim pretreatment, which is in agreement with our previous data, showing the anticonvulsant activity of diazoxide, as a K_{ATP} channel opener, against dichlorvas-induced seizure (5). In the current study, glibenclamide, as a potassium K_{ATP} channel blocker, reversed the anticonvulsant effects of cromakalim.

K^+ channels comprise a large family of ion channels. Among different types of K^+ channels, K_{ATP} channels are involved in numerous physiological functions (8). They are located pre- and postsynaptically in many brain areas, and their function is controlled by the metabolic conditions of neurons. They open and close in response to alterations in

Table 1. Effects of different doses of cromakalim and glibenclamide on the stages of seizure, induced by dichlorvos (50 mg/kg) and physostigmine (2 mg/kg). Data are shown as percentage in mice (n, 10). The definition of each stage is described in section 2.4.

Treatment	Organophosphate	Stage of Seizure		
		Stage 0	Stage 1 - 3	Stage 4 - 6
Cromakalim 0	Dichlorvos	0	0	100
	Physostigmine	0	0	100
Cromakalim 0.1	Dichlorvos	10	10	80
	Physostigmine	10	20	70
Cromakalim 10	Dichlorvos	40	20	40
	Physostigmine	50	20	30
Cromakalim 30	Dichlorvos	80	10	10
	Physostigmine	80	10	10
Glibenclamide 1	Dichlorvos	0	0	100
	Physostigmine	0	0	100
Glibenclamide 1 + cromakalim 30	Dichlorvos	0	10	90
	Physostigmine	10	10	80

Figure 1. Effects of Different Doses of Cromakalim (0.1, 10, and 30 μg/kg) and Glibenclamide (1 mg/kg) on the Onset of Seizure After Dichlorvos (50 mg/kg) and Physostigmine (2 mg/kg) Injection

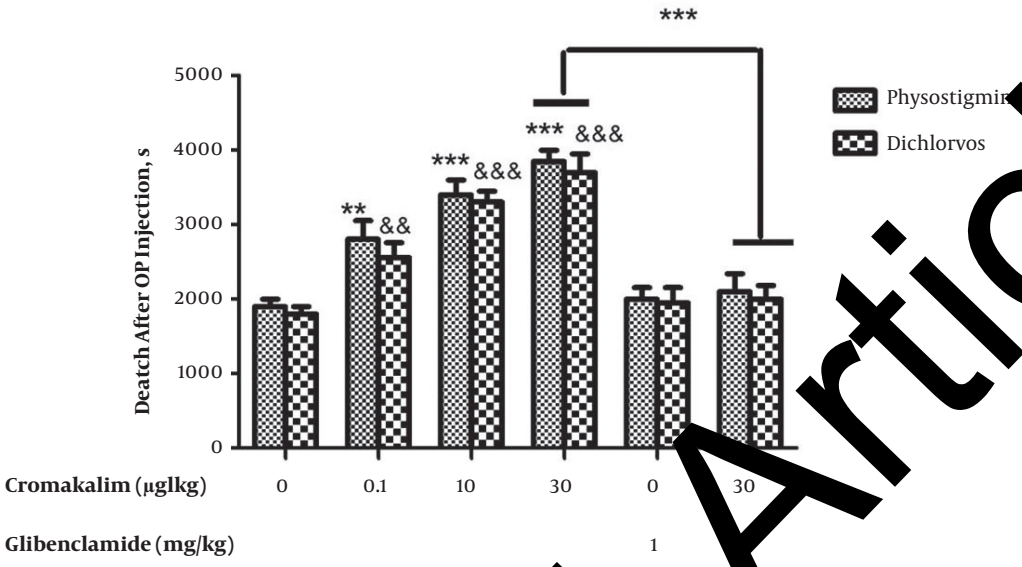


Effect of glibenclamide (1 mg/kg) and cromakalim (30 μg/kg) coadministration on the onset of seizure in mice. Values represent the mean and SEM of 10 animals. #P < 0.05, ##P < 0.01, compared to the physostigmine group (control); **P < 0.01, compared to the dichlorvos group (control); ***P< 0.001 compared to the cromakalim (1 mg/kg) plus dichlorvos group.

intracellular ATP/adenosine diphosphate (ADP) relations. Low ATP opens these channels, leading to K^+ efflux and cell hyperpolarization (24).

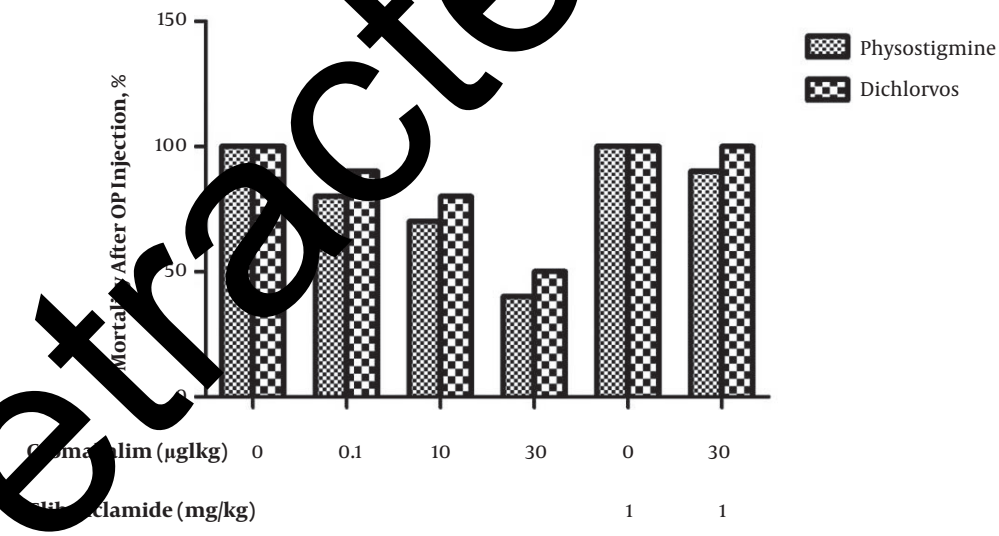
The hyperpolarization induced by K_{ATP} channel opening inhibits action-potential firing and prevents hypersynchronous neuronal discharge during a seizure. It has been shown that K_{ATP} channels play a significant role in the regulation of seizure threshold in several in vitro and in vivo models (13, 14, 25). In addition, K_{ATP} channel openers have been shown to decrease excitability in CA3 hippocampal

Figure 2. Effects of Different Doses of Cromakalim (0.1, 10, and 30 μg/kg) and Glibenclamide (1 mg/kg) on the Latency of Death After Dichlorvos (50 mg/kg) and Physostigmine (2 mg/kg) Injection



The effect of glibenclamide (1 mg/kg) and cromakalim (30 μg/kg) coadministration on the onset of death in mice. Values represent the mean and SEM of 10 animals. &&P < 0.01, &&&P < 0.001, compared to the dichlorvos group (control); **P < 0.01 and ***P < 0.001 compared to the physostigmine group (control); ***P < 0.001 compared to the cromakalim (1 mg/kg) plus dichlorvos group.

Figure 3. Effects of Different Doses of Cromakalim (0.1, 10, and 30 μg/kg) and Glibenclamide (1 mg/kg) on Mortality After Dichlorvos (50 mg/kg) and Physostigmine (2 mg/kg) Injection



Effects of glibenclamide (1 mg/kg) and cromakalim (30 μg/kg) coadministration on mortality in mice. Data are shown as percentage in mice (n, 10).

cells (26) and exhibit antiepileptic effects in a model of drug-induced epilepsy (27).

Molecular studies have shown that functional K_{ATP} channels are octomeric complexes, consisting of 4 inward

rectifier K^+ channel subunits (Kir6.1 or Kir6.2) and 4 sulfonylurea receptor subunits (SUR1, SUR2A, or SUR 2B), with diverse neurons expressing special combinations of K_{ATP} subunits (11). Mice without the expression of either SUR1 or Kir6.1 genes are susceptible to seizures (28). Moreover, it has been shown that mice with deficiencies in a subunit of K_{ATP} channels (Kir $6.2^{-/-}$ mice) are vulnerable to generalized seizure (14). In addition, it has been recently reported that K_{ATP} channel openers, such as cromakalim and diazoxide, increase clonic seizures induced by pentylenetetrazole in mice (9, 29).

The role of K channel openers in seizure has been shown in several clinical and animal studies. However, the effect of these substances on seizures induced by OPs and carbamates has not been studied yet; also, there is no article showing the possible effects. In this study, we showed that cromakalim (a K_{ATP} channel opener) at doses of 0.1, 10, and 30 μg/kg could reverse convulsion and death following OP/carbamate administration. In addition, our data showed that the antiepileptic effect of cromakalim is reversed by glibenclamide (a K^+ channel blocker). These data confirm the role of K channels in mediating the convulsive effects of OPs and carbamates. These findings help introduce new aspects of specific targets to manage patients with OP or carbamate toxicity.

In summary, this study, for the first time, showed that cromakalim (a K_{ATP} channel opener) decreases seizures induced by dichlorvos and physostigmine in mice. We also introduced new aspects of specific targets to manage seizures from OP/carbamate toxicity, although further investigation is needed to evaluate the efficacy of these agents in AChEI-induced seizures.

Footnote

Authors' Contributions: Study concept and design: Abbas Norouzi-Javidan, Sattar Ostadhadi, and Ahmad-Reza Dehpour; acquisition of data: Sattar Ostadhadi, Abouzar Moradi, and Samira Zolfaghari; interpretation of data: Abbas Norouzi-Javidan and Ahmad-Reza Dehpour; drafting of the manuscript: Sattar Ostadhadi and Samira Zolfaghari; critical revision of the manuscript for important intellectual content: Sattar Ostadhadi, Ahmad-Reza Dehpour, Samira Zolfaghari, Abouzar Moradi, and Abbas Norouzi-Javidan; statistical analysis: Sattar Ostadhadi and Samira Zolfaghari.

References

1. Cocker J, Mason HJ, Garfitt SJ, Jones K. Biological monitoring of exposure to organophosphate pesticides. *Toxicol Lett*. 2002;**134**(1-3):97-103. [PubMed: 12191866].
2. Millard CB, Broomfield CA. Anticholinesterases: medical applications of neurochemical principles. *J Neurochem*. 1995;**64**(5):1909-18. [PubMed: 7722478].
3. Fukuto TR. Mechanism of action of organophosphorus and carbamate insecticides. *Environ Health Perspect*. 1990;**87**:245-54. [PubMed: 2176588].
4. Cable GG, Doherty S. Acute carbamate and organochlorine toxicity causing convulsions in an agricultural pilot: a case report. *Aviat Space Environ Med*. 1999;**70**(1):68-72. [PubMed: 9895024].
5. Jazayeri A, Zolfaghari S, Ostadhadi S. Anticonvulsant effect of Diazoxide against Dichlorvos-induced seizures in mice. *Artificial Cells J*. 2013;**2013**:697305. doi: 10.1155/2013/697305. [PubMed: 24453...].
6. Lallement G, Dorandeu F, Filliat..., Carpentier P, Baille V, Blanchet G. Medical management of organophosphate-induced seizures. *J Physiol Paris*. 1998;**92**(5-6):369-73. doi: 10.1016/S09...80007-2. [PubMed: 9789839].
7. Munro NB, Watson AP, Amb... KR, Griffin GD. Treating exposure to chemical warfare agents: implications for health care providers and community emergency planning. *Environ Health Perspect*. 1990;**89**:205-15. [PubMed: 2088748].
8. Yamada K, Inagaki N. Neuroprotection by KATP channels. *J Mol Cell Cardiol*. 2005;**38**(...-9. doi: 10.1016/j.yjmcc.2004.11.020. [PubMed: 15910879].
9. Shafaroodi H, Asa..., Sadegh...pour H, Ghasemi M, Ebrahimi F, Tavakoli S, et al. Role of ATP-sensitive potassium channels in the biphasic effects of morphine on pentylenetetrazole-induced seizure threshold in mice. *Epilepsy Res*. 2007;**75**(1):63-9. doi: 10.1016/j.eplepsyres.2007.04.005. [PubMed: 17517498].
10. ...emi M, Shafaroodi H, Karimollah AR, Gholipour T, Nezami BG, Ebra...mi F, et al. ATP-sensitive potassium channels contribute to the ti...dependent alteration in the pentylenetetrazole-induced seizu... threshold in diabetic mice. *Seizure*. 2010;**19**(1):53-8. doi: ...6/j.seizure.2009.11.003. [PubMed: 20004596].
11. Wickenden AD. Potassium channels as anti-epileptic drug targets. *Neuropharmacology*. 2002;**43**(7):1055-60. [PubMed: 12504910].
12. Porter RJ, Rogawski MA. New antiepileptic drugs: from serendipity to rational discovery. *Epilepsia*. 1992;**33 Suppl 1**:1-6. [PubMed: 1379532].
13. Narita M, Suzuki T, Misawa M, Nagase H, Nabeshima A, Ashizawa T, et al. Role of central ATP-sensitive potassium channels in the analgesic effect and spinal noradrenaline turnover-enhancing effect of intracerebroventricularly injected morphine in mice. *Brain Res*. 1992;**596**(1-2):209-14. [PubMed: 1467984].
14. Yamada K, Ji JJ, Yuan H, Miki T, Sato S, Horimoto N, et al. Protective role of ATP-sensitive potassium channels in hypoxia-induced generalized seizure. *Science*. 2001;**292**(5521):1543-6. doi: 10.1126/science.1059829. [PubMed: 11375491].
15. Singh NA, Charlier C, Stauffer D, DuPont BR, Leach RJ, Melis R, et al. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. *Nat Genet*. 1998;**18**(1):25-9. doi: 10.1038/ng0198-25. [PubMed: 9425895].
16. Dekundy A, Kaminski RM, Turski WA. Dizocilpine improves beneficial effects of cholinergic antagonists in anticholinesterase-treated mice. *Toxicol Sci*. 2003;**72**(2):289-95. doi: 10.1093/toxsci/kfg013. [PubMed: 12660363].
17. McLean MJ, Gupta RC, Dettbarn WD, Wamil AW. Prophylactic and therapeutic efficacy of memantine against seizures produced by soman in the rat. *Toxicol Appl Pharmacol*. 1992;**112**(1):95-103. [PubMed: 1733053].
18. Tattersall J. Seizure activity post organophosphate exposure. *Front Biosci (Landmark Ed)*. 2009;**14**:3688-711. [PubMed: 19273303].
19. Dekundy A, Kaminski RM, Zielinska E, Turski WA. NMDA antagonists exert distinct effects in experimental organophosphate or carbamate poisoning in mice. *Toxicol Appl Pharmacol*. 2007;**219**(2-3):114-21. doi: 10.1016/j.taap.2006.10.030. [PubMed: 17157343].

20. Milatovic D, Gupta RC, Aschner M. Anticholinesterase toxicity and oxidative stress. *ScientificWorldJournal*. 2006;**6**:295–310. doi: 10.1100/tsw.2006.38. [PubMed: 16518518].
21. Sparenborg S, Brennecke LH, Jaax NK, Braitman DJ. Dizocilpine (MK-801) arrests status epilepticus and prevents brain damage induced by soman. *Neuropharmacology*. 1992;**31**(4):357–68. [PubMed: 1522953].
22. Bowden CA, Krenzelok EP. Clinical applications of commonly used contemporary antidotes. A US perspective. *Drug Saf*. 1997;**16**(1):9–47. [PubMed: 9010641].
23. Clement JG. Central activity of acetylcholinesterase oxime reactivators. *Toxicol Appl Pharmacol*. 1992;**112**(1):104–9. [PubMed: 1733041].
24. de Weille JR, Lazdunski M. Regulation of the ATP-sensitive potassium channel. *Ion Channels*. 1990;**2**:205–22. [PubMed: 2102815].
25. Katsumori H, Ito Y, Higashida H, Hashii M, Minabe Y. Anti and proconvulsive actions of levcromakalim, an opener of ATP-sensitive K+ channel, in the model of hippocampus generating partial seizures in rats. *Eur J Pharmacol*. 1996;**311**(1):37–44. doi: 10.1016/0014-2999(96)00400-1.
26. Alzheimer C, ten Bruggencate G. Actions of BRL 34915 (Cromakalim) upon convulsive discharges in guinea pig hippocampal slices. *Naunyn Schmiedebergs Arch Pharmacol*. 1988;**337**(4):429–34. [PubMed: 3405317].
27. Gandolfo G, Gottesmann C, Bidard JN, Lazdunski M. Subtypes of K+ channels differentiated by the effect of K+ channel openers upo K+ channel blocker-induced seizures. *Brain Res*. 1989;**495**(1):189– [PubMed: 2550110].
28. Ben-Ari Y, Cossart R. Kainate, a double agent that generate izure two decades of progress. *Trends Neurosci*. 2000;**23**(11):580–7. [P ed: 11074268].
29. Niaki SE, Shafaroodi H, Ghasemi M, Shakiba B, Fakh i A, Dehpour A Mouth breathing increases the pent etetrazo induced se ure threshold in mice: a role for ATP-sensitive pot ium cha els. *Epilepsy Behav*. 2008;**13**(2):284–9. doi: 10. i.yeb .013. [PubMed: 18508411].