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RESEARCH ARTICLE

Effects of aconitine on membrane currents and action potentials in neonatal rat ventricular myocytes and its impact on electrocardiographic changes

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ABSTRACT

This study presents an analysis of the electrophysiological effects of aconitine, a toxic diterpenoid alkaloid derived from Aconitum plants, on neonatal rat ventricular myocytes (NRVMs). The research investigates the potential impact of aconitine on various ion currents and cardiac action potentials, shedding light on its arrhythmogenic properties. Whole-cell patch-clamp experiments were conducted to assess the effects of aconitine on delayed-rectifier K+ currents ($I_{K(DR)}$) and inwardly rectifying K+ currents ($I_{K(IR)}$) in NRVMs. The findings indicate that aconitine exposure led to the inhibition of ($I_{K(DR)}$) and ($I_{K(IR)}$), suggesting its potential influence on cardiac repolarization and excitability. Notably, aconitine induced transient inward current (I_{TI}) and early after-depolarizations (EADs) in a concentration-dependent manner, both of which have implications for cardiac arrhythmias. Moreover, the study examined the electrocardiogram changes in Sprague-Dawley rats upon aconitine injection, revealing a prolonged QT interval and the emergence of polymorphic ventricular tachycardia (VTs), indicative of arrhythmic effects. The study emphasizes the importance of understanding the electrophysiological impact of aconitine and similar compounds, considering their potential therapeutic applications and associated toxicities.

Introduction

We recently had the pleasure of reading an informative review article titled "A Review on Efforts for Improvement In Medicinally Important Chemical Constituents In *Aconitum* Through Biotechnological Interventions" by Tiwari et al. [1], which appeared in 3 Biotech (https://www.springer.com/journal/13205). The research described in the article highlights the medicinal value and pharmacological activities of aconitine and other structurally similar compounds, as demonstrated previously [2,3,4,5]. Aconitine, a highly toxic diterpenoid alkaloid derived from the plant species *Aconitum*, has been recognized for its potential phytomedical effects on the heart, despite its significant toxicological properties [6-9]. Therefore, it is important to understand more thoroughly the electrophysiological effect of Aconitine in both cellular and clinical level. In our recent study, we conducted a series of experiments using the state-of-the-art whole-cell patch-clamp technique to investigate the electrophysiological impact of aconitine on neonatal rat ventricular myocytes (NRVMs).

Materials and Methods

ISOLATION AND CULTURE OF NEONATAL RAT VENTRICULAR MYOCYTES

This study employed the method of Wang et al, So et al., and the methods description partly reproduces their wording [10,11]. Cells were isolated from 1- to 2-day-old Sprague-Dawley rats by enzymatic digestion with 0.1% trypsin and 0.03% collagenase. After isolation, cells were plated onto laminin-coated 35–mm dishes at a density of 1x 10^3 cells/mm^2 and cultured for 48 h in the medium of Dulbecco's modified Eagle's medium and Medium 199 (4:1) containing 10% fetal calf serum, 4mM L-glutamine, 100 units/ml penicillin/streptomycin, and 0.1mM 5-bromo-2-deoxyuridine. 5-Bromo-2- deoxyuridine was used to inhibit fibroblast proliferation. The animal experiments were conducted according to protocols that follow the National Institutes of Health standards and the guidelines for the Care and Use of Experimental Animals. The procedure was approved by the Animal Care and Use Committee of the National Cheng Kung University, Taiwan

WHOLE-CELL PATCH-CLAMP RECORDINGS

The technique and preparation procedures resemble our previous study by Foon et al., and our wording used partly resembled their descriptions. [12] Cells used for experiments were dissociated, and an aliquot of cell suspension was transferred to a recording chamber mounted on the stage of an inverted DM-IL microscope (Leica Microsystems, Wetzlar, Germany). Neonatal rat ventricular myocytes were bathed at room temperature (20–25°C) in normal Tyrode's solution containing 1.8mM CaCl2. Patch pipettes were pulled from Kimax-51 glass capillary tubes (Kimble Glass; Vineland, NJ) using a two-stage electrode puller (PP-830; Narishige, Tokyo, Japan), and the tips were fire polished with a microforge (MF-83; Narishige). The pipettes used had resistances of 3–5 MX when immersed in normal Tyrode's solution. Ion currents were measured in the whole-cell mode of the patch-clamp recordings with an RK-400 (Biologic, Claix, France) or an Axopatch 200B patch-clamp amplifier (Molecular Devices, Sunnyvale, CA) [13,14] The signals were displayed on an HM-507 oscilloscope (Hameg, East Meadow, NY) and on a Dell 2407WFP-HC LCD monitor (Round Rock, TX). The data were stored online in a Slimnote VX3 computer (Lemel, Taipei, Taiwan) via a universal serial bus port at 10 kHz through a Digidata-1322A interface (Molecular Devices). This device was controlled by pCLAMP 9.0 software (Molecular Devices). Currents were low-pass filtered at 1 or 3 kHz. Ion currents recorded during whole-cell experiments were digitally stored and analyzed subsequently by use of pCLAMP 9.0, Origin 7.5 software (OriginLab, Northampton, MA) or custom-made macros in Microsoft Excel (Redmont, WA).

Results

EFFECT OF ACONITINE ON DELAYED-RECTIFIER K+ CURRENT (*IK*(DR)) IN NEONTAL RAT VENTRICULAR MYOCYTES(NRVMs).

As illustrated in **Figure 1a**, it was observed from whole-cell current recordings that NRVM exposure to aconitine (1-10 μM) resulted in a direct inhibition of delayed-rectifier K^+ currents ($I_{K(DR)}$), accompanied by a concurrent increase in current inactivation, as described previously[10,15]. The inactivation time course of $I_{K(DR)}$ in response to membrane depolarization was progressively increased in the presence of aconitine. When a series of depolarizing voltage pulses was applied to the tested cell, it was consistently observed that the magnitude of peak $I_{K(DR)}$ was suppressed to a less extent compared to that of sustained $I_{K(DR)}$ (**Figure 1b**).

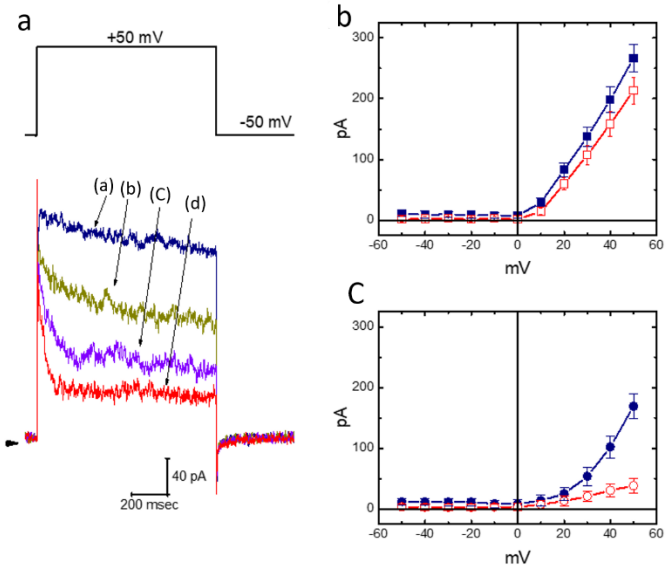

Figure 1: Effect of aconitine on delayed-rectifier K^+ current ($I_{K(DR)}$) in neonatal rat ventricular myocytes (NRVMs).

NRVMs were isolated from 1 to 2-day-old Sprague-Dawley rats by enzymatic digestion with 0.1% trypsin and 0.03% collagenase. In this set of whole-cell measurements, the cells were placed in Ca^{2+}-free Tyrode's solution containing 1μM tetrodotoxin and 0.5 mM $CdCl_2$. The recording pipette was filled with a K^+-enriched solution, and whole-cell current recordings were performed. **a.** Representative current traces obtained in the control period **(a)**, aconitine was not present), and during cell exposure to 1μM aconitine **(b)**, 3μM aconitine **(c)**, or 10μM aconitine**(d)**.

The applied voltage clamp protocol is depicted in the upper part. The arrowhead in the lower left corner indicates the zero current level, while the calibration bar in the lower right corner is applied to all current traces provided. Averaged current versus voltage relationships of $I_{K(DR)}$ obtained in the control (**b,** filled square symbols) and during the exposure to 10μM aconitine (**c,** open circle symbols). Filled or open symbols in both **b** and **c** were respectively taken at the beginning or end of each depolarizing pulse. Each point in **b** and **c** indicates the mean ± SEM (n=9). Please note that the inhibitory effect of aconitine on sustained $I_{K(DR)}$ in NRVMs is stronger than its effect on peak $I_{K(DR)}$.

EFFECT OF ACONITINE ON INWARDLY RECTIFYING K^+ CURRENT ($I_{K(IR)}$) IN NRVMs.

Aconitine at concentration of 1 and 10 μM was also found to suppress the amplitude of the inwardly rectify K^+ currents ($I_{K(IR)}$) elicited by a long-lasting ramp pulse (**Figures 2a and 2b**). In the continued presence of 10 μM aconitine, the addition of 1 mM $BaCl_2$ completely abolished the amplitude of this current, as shown in **Figure 2b**.

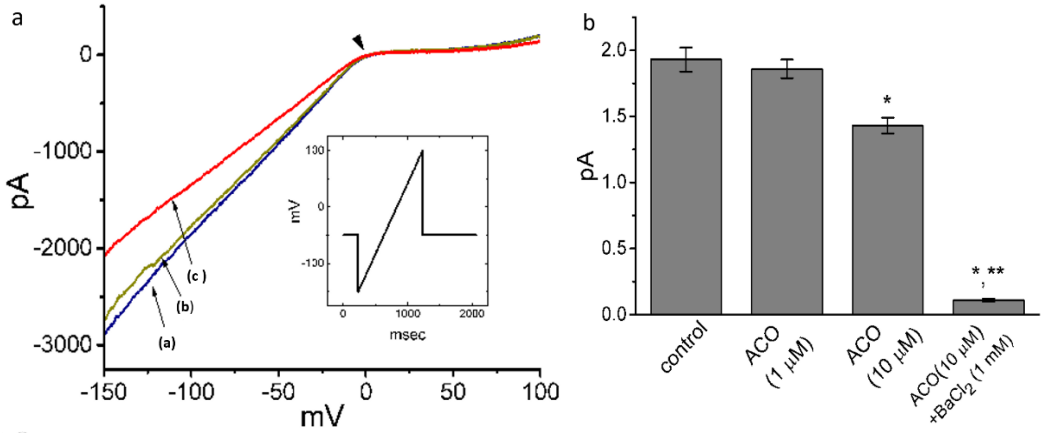

Figure 2: Effect of aconitine on inwardly rectifying K^+ current ($I_{K(IR)}$) in NRVMs.

The experiments were conducted in cells bathed in a high-K^+, Ca^{2+}-free solution, and the measuring pipette was filled with K^+-enriched solution. **a** Representative current traces elicited by a long-lasting ramp pulse (indicated in inset). The arrowhead indicates an approximate reversal potential of 0 mV. (a): control; (b): 1μM aconitine, c: 10μM aconitine. **b** Summary of the data showing inhibitory effects of aconitine (ACO) and aconitine plus $BaCl_2$ on $I_{K(IR)}$ amplitude (mean ± SEM; n = 9 for each gray bar). The amplitude of $I_{K(IR)}$ was measured at a voltage level of -100 mV. *Significantly different from control ($P<0.05$) and **significantly different from aconitine (10μM) alone group ($P<0.05$).

EFFECT OF ACONITINE ON THE INDUCTION OF TRANSIENT INWARD CURRENT (I_{TI}) RECORDED FROM NRVMs.

Furthermore, when the tested cell was continually exposed to 10 μM aconitine, a downward deflection of inward current was observed after returning to -50 mV following depolarization from -50 to 0 mV for a duration of 300 ms. This phenomenon, referred to as transient inward current (I_{TI}), is depicted in **Figure 3a and 3b**. When the concentration was increased to 30 μM, both the amplitude and frequency of I_{TI} obtained observed after repolarization to -50 mV were progressively enhanced (**Figure 3a**). The I_{TI} in heart cells can induce delayed afterdepolarizations (DADs). DADs are abnormal depolarizations that occur during the repolarization phase of the cardiac action potential. Upon further increasing the concentration of aconitine to 100 μM, the spontaneous activity of I_{TI} with irregular magnitude and frequency was observed at a voltage level of -50 mV was found to emerge (**Figure 3b**). Similar findings were observed across seven different cells.

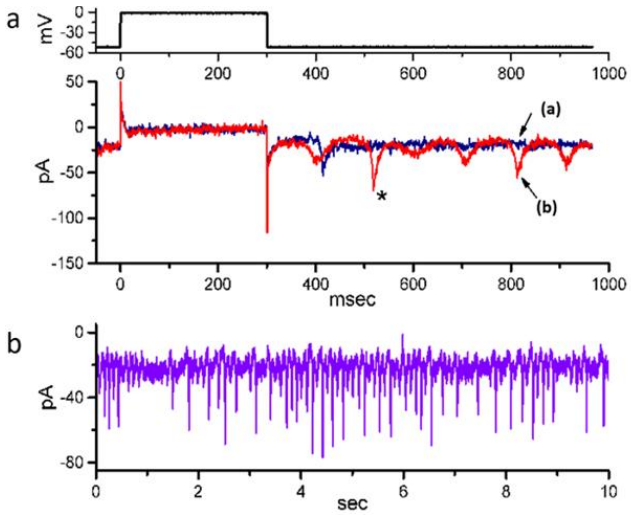

Figure 3: Effect of aconitine on the induction of transient inward current (I_{TI}) recorded from NRVMs.

In these experiments, NRVMs were bathed in HEPES-buffered normal Tyrode's solution containing 1.8 mM $CaCl_2$, and whole-cell current recordings were conducted using a K^+-containing solution-filled pipette. In **a**, current traces were elicited to 0 mV from a holding potential of -50 mV (indicated in the upper part). Importantly, after the potential returned to -50 mV, multiple forms of inward currents (i.e., I_{TI}) (indicated by asterisk) appeared. **(a)**: 10 μM aconitine (black color); **(b)**: 30 μM aconitine (red color). **b** Repetitive occurrence of I_{TI} with irregular amplitude observed during cell exposure 100 μM aconitine. In these experiments, the NRVM under investigation was held at the level of -50 mV, and whole-cell current recording was performed. The downward deflection indicates the presence of I_{TI}, which corresponds to triggered activity mediated by delayed afterdepolarization (DAD) observed in current-clamp mode.

EFFECT OF ACONITINE ON ACTION POTNETIALS (APs) RECORDED FROM NRVMs.

Whole-cell potential recordings were also conducted to determine possible effects of aconitine on membrane potential changes found in NRVMs. As depicted in **Figure 4**, the cells were bathed in a normal Tyrode's solution containing 1.8 mM $CaCl_2$, while the pipette was filled with a K^+-containing solution for current-clamp configuration. Upon exposure to 4 μM aconitine, the duration of cardiac action potential was observed to be prolonged. Moreover, with an increase in aconitine concentration to 10 and 30 μM, the progressive prolongation of action potential accompanied by the emergence of early after-depolarizations

(EADs) was noted [15]. These consistent findings were replicated in eight different cells. EADs are abnormal electrical events that can occur during the repolarization phase of a cardiac action potential. During the presence of aconitine, the repolarization phase can be seriously retarded, leading to the development of EADs.

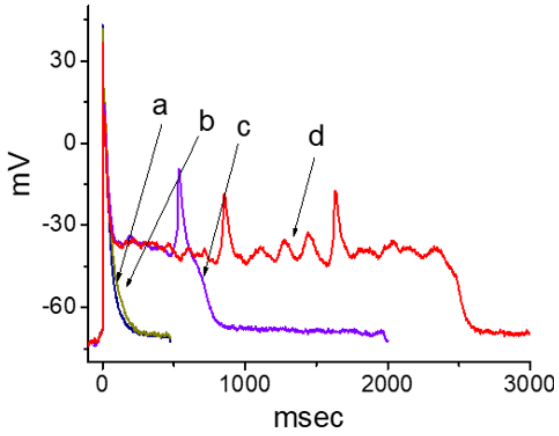

Figure 4: Effect of aconitine on action potentials (APs) recorded from NRVMs.

AP recordings were obtained from NRVMs using the whole-cell current-clamp configuration. The cells were immersed in normal Tyrode's solution containing 1.8 mM CaCl2, and the measuring pipette was filled with a K^+-containing solution. The holding current was set at 0 nA, and the potential was recorded. The original potential trace labelled "a" represents the control period without the presence of aconitine. Traces labelled "b", "c", and "d" correspond to recordings obtained after the addition of 3μM, 10μM, and 30μM aconitine, respectively. Notably, concentration of aconitine exceeding 10μM led to a marked prolongation of APs, accompanied by the emergence of early-after depolarizations (EADs).

ELECTROCARDIOGRAPHIC CHANGES IN A SPRAGUE-DAWLEY RAT (325 g) DURING THE CONTROL PERIOD (a, ACONITINE WAS NOT PRESENT) AND 10 min AFTER PERITONEAL INJECTION OF ACONITINE (b, 400 μg/kg).

In a final set of experiments, we recorded changes in the electrocardiogram (ECG) of Sprague-Dawley rats. One minute after the peritoneal injection with aconitine (400 μg/Kg), we noticed a gradual prolongation of the QT interval from the changes in the electrocardiogram. As depicted in **Figure 5**, ten minutes after intraperitoneal injection of 400 μg/Kg aconitine, the emergence of polymorphic ventricular tachycardia (VTs) was progressively observed, as described previously [6,8,9,16]. The Morphological characteristics of waveforms in VTs vary greatly. These findings were consistent across eight different rats. Polymorphic VY refers to an abnormal heart rhythm characterized by a rapid and irregular ventricular heartbeat. In this condition, the QRS complexes on the electrocardiogram display varying morphologies, meaning that the shape and amplitude of the QRS complexes change from beat to beat.

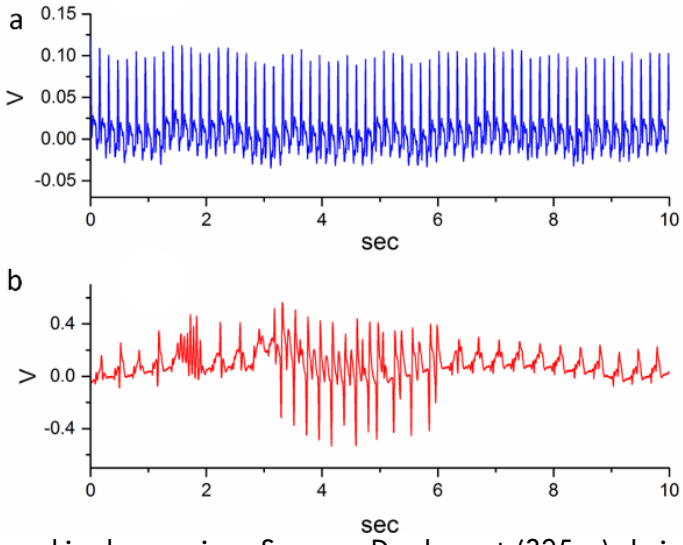

Figure 5 Electrocardiographic changes in a Sprague-Dawley rat (325 g) during the control period (a, aconitine was not present) and 10 min after peritoneal injection of aconitine (b, 400 μg/kg).

The electrocardiographic signals were recorded from anesthetized Sprague-Dawley rats weighing 350-400 g, which were fed a standard diet and given water ad libitum. In **b**, the waveform indicates the presence of polymorphic ventricular tachycardia (VT), characterized by a large and irregular magnitude. Note that there is a distinct difference in the vertical scales between **a** and **b**.

Discussion

This research delves into the electrophysiological effects of aconitine, a toxic diterpenoid alkaloid derived from Aconitum plants, on neonatal rat ventricular myocytes (NRVMs). The investigation explores how aconitine may influence various ion currents and cardiac action potentials, providing insights into its arrhythmogenic properties. Utilizing whole-cell patch-clamp experiments, we evaluated the impact of aconitine on delayed-rectifier K^+ currents ($I_{K(DR)}$) and inwardly rectifying K+ currents ($I_{K(IR)}$) in NRVMs. The results suggest that aconitine exposure inhibited both ($I_{K(DR)}$) and ($I_{K(IR)}$), indicating its potential influence on cardiac repolarization and excitability. Notably, aconitine induced transient inward currents (I_{TI}) and early after-depolarizations (EADs) in a concentration-dependent manner, both of which bear significance for cardiac arrhythmias. Additionally, the study investigated electrocardiogram (ECG) changes in Sprague-Dawley rats following aconitine injection, revealing a prolonged QT interval and the onset of polymorphic ventricular tachycardia (VTs), indicative of arrhythmic effects. The research underscores the importance of comprehending the electrophysiological impact of aconitine and similar compounds, considering their potential therapeutic applications and associated toxicities.

Aconitine is recognized one of the most toxic plant alkaloids known, and its poisoning can manifest rapidly and severely, necessitating immediate medical attention upon suspected exposure. Its cardiac toxicity can precipitate swift and profound cardiovascular collapse, potentially culminating in cardiac arrest and fatality without prompt intervention. Aconitine toxicity can prompt several cardiac arrhythmias as presented herein, which are aberrant heart rhythms that can profoundly compromise the heart function. The ionic mechanism underlying the arrhythmogenic effects of aconitine involves its stimulation of voltage-gates Na^+ current and suppression voltage-gated K^+ current, along with an acceleration in the inactivation time course [9,10,15]

Conclusions

The presence of aconitine can induce arrhythmogenic effects when combined with the findings of this study and the results of previous studies[6,7] [8,9,16]. Aconitine has also been shown to block hERG channels [17]. Therefore, this class of compounds can indeed cause cardiac arrhythmias at a certain concentration. It is necessary to have a thorough understanding of the potential effects on cardiac cell currents and potentials before assessing the therapeutic use of aconitine or structurally similar compounds e.g., oxonitine, hypaconitine, mesaconitine, deoxyaconitine, and lappaconitine. Before assessing the therapeutic (e.g., antitumor) effects of these *Aconitum* extracts [4,5], the potential toxicities associated with these compounds need to be carefully considered.

Data Availability

The original data is available on reasonable request from the corresponding author.

Conflicts of Interest

"The authors declared that there is no conflict of interest regarding the publication of this paper."

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References

1. Tiwari S, Acharya P, Solanki B, Sharma AK, Rawat S. A review on efforts for improvement in medicinally important chemical constituents inAconitum through biotechnological interventions. *3 Biotech*. Jun 2023;13(6):190. doi:10.1007/s13205-023-03578-z
2. Chan TY. Aconitum Alkaloid Poisoning Because of Contamination of Herbs by Aconite Roots. *Phytother Res*. Jan 2016;30(1):3-8. doi:10.1002/ptr.5495
3. Jin X, Cheng J, Zhang Q, et al. Aconitine - A promising candidate for treating cold and mechanical allodynia in cancer induced bone pain. *Biomed Pharmacother*. May 2023;161:114284. doi:10.1016/j.biopha.2023.114284
4. Salehi A, Ghanadian M, Zolfaghari B, et al. Neuropharmacological Potential of Diterpenoid Alkaloids. *Pharmaceuticals (Basel)*. May 14 2023;16(5) doi:10.3390/ph16050747
5. Xiang G, Xing N, Wang S, Zhang Y. Antitumor effects and potential mechanisms of aconitine based on preclinical studies: an updated systematic review and meta-analysis. *Front Pharmacol*. 2023;14:1172939. doi:10.3389/fphar.2023.1172939
6. Yeih DF, Chiang FT, Huang SK. Successful treatment of aconitine induced life threatening ventricular tachyarrhythmia with amiodarone. *Heart*. Oct 2000;84(4):E8 . doi:10.1136/heart.84.4.e8
7. Chan TY. Aconite poisoning. *Clin Toxicol (Phila)*. Apr 2009;47(4):279-85. doi:10.1080/15563650902904407
8. Kitamura T, Fukamizu S, Hojo R, et al. Various morphologies of bidirectional ventricular tachycardia caused by aconite "Torikabuto" poisoning. *J Cardiol Cases*. Feb 2013;7(2):e42-e44. doi:10.1016/j.jccase.2012.10.004
9. Coulson JM, Caparrotta TM, Thompson JP. The management of ventricular dysrhythmia in aconite poisoning. *Clin Toxicol (Phila)*. Jun 2017;55(5):313-321. doi:10.1080/15563650.2017.1291944
10. Lin MW, Wang YJ, Liu SI, Lin AA, Lo YC, Wu SN. Characterization of aconitine-induced block of delayed rectifier K+ current in differentiated NG108-15 neuronal cells. *Neuropharmacology*. May 2008;54(6):912-23. doi:10.1016/j.neuropharm.2008.01.009
11. So EC, Liu PY, Lee CC, Wu SN. High Effectiveness in Actions of Carfilzomib on Delayed-Rectifier K(+) Current and on Spontaneous Action Potentials. *Front Pharmacol*. 2019;10:1163. doi:10.3389/fphar.2019.01163
12. Foo NP, Liu YF, Wu PC, Hsing CH, Huang BM, So EC. Midazolam's Effects on Delayed-Rectifier K(+) Current and Intermediate-Conductance Ca(2+)-Activated K(+) Channel in Jurkat T-lymphocytes. *Int J Mol Sci*. Jul 4 2021;22(13)doi:10.3390/ijms22137198
13. Wang Y, Cheng J, Tandan S, Jiang M, McCloskey DT, Hill JA. Transient-outward K+ channel inhibition facilitates L-type Ca2+ current in heart. *J Cardiovasc Electrophysiol*. Mar 2006;17(3):298-304. doi:10.1111/j.1540-8167.2006.00362.x
14. Wu SN, Chang HD, Sung RJ. Cocaine-induced inhibition of ATP-sensitive K+ channels in rat ventricular myocytes and in heart-derived H9c2 cells. *Basic Clin Pharmacol Toxicol*. May 2006;98(5):510-7. doi:10.1111/j.1742-7843.2006.pto_354.x
15. Wang YJ, Chen BS, Lin MW, et al. Time-dependent block of ultrarapid-delayed rectifier K+ currents by aconitine, a potent cardiotoxin, in heart-derived H9c2 myoblasts and in neonatal rat ventricular myocytes. *Toxicol Sci*. Dec 2008;106(2):454-63. doi:10.1093/toxsci/kfn189
16. Zhao YT, Wang L, Yi Z. An Unusual Etiology for Bidirectional Ventricular Tachycardia. *Can J Cardiol*. Mar 2016;32(3):395 e5-6. doi:10.1016/j.cjca.2015.06.024
17. 1Kiss T, Borcsa B, Orvos P, Talosi L, Hohmann J, Csupor D. Diterpene Lipo-Alkaloids with Selective Activities on Cardiac K+ Channels. *Planta Med*. Nov 2017;83(17):1321-1328. doi:10.1055/s-0043-109556