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EFFECT OF ANTI-ARRHYTHMIC AGENTS ON SODIUM CALCIUM EXCHANGER IN THE FROG HEART

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ABSTRACT

Background and Objectives: In amphibian ventricle, steady loss of calcium occurs during diastole. Sodium calcium exchanger (NCX) clears calcium to the exterior causing rhythm generation. Calcium loss was demonstrated by decrease in the contractile force after an imposed rest period-rest induced decay (RID). Calcium channel blockers with anti-arrhythmic activity cause increase in contractile force following a rest period (rest induced potentiation), which could be due to reversal of NCX. Hence other therapeutically used anti-arrhythmic agents may also influence the working direction of NCX. **Materials and Methods:** Frog ventricular strips when electrically stimulated in solution resembling extracellular fluid show a steady state of contraction. Different classes of anti-arrhythmic agents according to Vaugan William's classification belonging to classes Ia, Ib, III and IV were examined for reversal of NCX. Rest periods were imposed in between contraction, ranging from 20 to 100 and 180 s and similar protocol was followed with the drug of intervention on the same tissue. The post rest and pre rest amplitudes of contraction were compared and analysed. **Results:** RID that was observed with the control solution did not get converted into potentiation with any of the above mentioned classes of anti-arrhythmic agents. **Interpretation and Conclusion:** Unlike the results observed with calcium channel blockers verapamil and diltiazem, no reversal in the direction of the working mode of NCX was noted with the above said anti-arrhythmic agents as shown by the persistence of RID. Hence these anti-arrhythmic agents do not influence the working mode of NCX in their anti-arrhythmic action.

Key words: Anti-arrhythmic, Calcium, Sodium calcium exchanger, Rest induced decay, Rest induced potentiation

INTRODUCTION

In amphibian ventricular muscle, there is a steady loss of calcium from sarcoplasmic reticulum (SR) during diastole accounting for what is referred to as rest induced decay (RID).[1] This phenomenon of RID could be experimentally demonstrated on imposing a period of rest in a steadily beating ventricular tissue. The decay in the amplitude of contraction may represent refilling of SR calcium stores that have become partially depleted of calcium during the rest, the longer the rest interval the lower the SR calcium content. This may be related to the rest decay of tension of the first post rest beat, since this is thought to be SR dependent.[2]

The calcium transporter on the SR causing its diastolic release of calcium is proposed to be the ryanodine receptor itself. Ryanodine receptor calcium release during the diastolic depolarisation produces a localized subsarcolemmal calcium increase which is responsible for the calcium induced calcium release from the SR.[3]

It has been proposed that the calcium transporter on the plasma membrane which clears the SR calcium released in diastole to the exterior is the sodium calcium exchanger (NCX).[4] Extrusion of calcium via NCX is responsible for producing an inward depolarizing current which is important rhythm generating mechanism. Reversal of the working direction of NCX based on the concentration gradient of ions across the sarcolemma may influence the diastolic generation of rhythm adversely. The working mode of NCX can be dynamically changed by altering [Ca]I over the physiological range and that outward I(NCX) can be activated quite rapidly with SR Ca release.[5]

Diastolic calcium extrusion through the NCX is said to play a role in rhythm generating mechanism in human heart, because the stoichiometry of the exchange of NCX is 3 Na^+: 1 Ca^{++}. The net inward depolarizing current is therefore involved in rhythm generation. Reversal of NCX results in SR acquiring calcium during diastole, which has been demonstrated as augmentation in the post rest amplitude of the contractile force. Hence reversal of NCX by pharmacological agents must therefore serve as an anti-arrhythmic intervention.

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In the frog ventricle, it has been shown that verapamil and diltiazem which are known calcium channel blockers with anti-arrhythmic property, when employed in the rest protocol experiments, instead of causing a decay in the contractile force, caused potentiation of post rest beat-which is referred to as rest induced potentiation (RIP).[6] Similar phenomenon has been demonstrated with interventions like low extracellular fluid (ECF) sodium, high ECF calcium and ouabain, all of them causing a reversal of the working mode of NCX.

Nifedepine, though a calcium channel blocker is not therapeutically employed in treating arrhythmias and interestingly does not produce RIP. Hence it has been suggested that drugs like verapamil and diltiazem, other than their conventional calcium channel blocking action on the L-type calcium channels on the sarcolemma may also block the diastolic leak of SR calcium or may add calcium to the SR during diastole.[6]

Sodium calcium exchanger is thus implicated in arrhythmogenesis. Hence it has been suggested that development of better anti-arrhythmic agent is possible by targeting the NCX.[7] Based on the above observation we propose that any event or drug that would promote the leak of calcium during diastole is potentially arrhythmogenic and that any intervention or drug that would prevent the diastolic leak of calcium could serve therapeutically as an anti-arrhythmic agent. In this study, different classes of anti-arrhythmic agents in clinical use are tested for their effect on NCX using the post rest force change as an indicator of the direction of NCX.

MATERIALS AND METHODS

Medium sized frogs weighing between 80 and 120 g belonging to the species *Rana hexadactyla* of both sexes were used for the study. The animals were housed in the breeding pond in the institution and were supplied with regular feeds. Guidelines formulated by the institution in anaesthetizing, usage and disposal of the animal were strictly adhered to during the research work. Hearts isolated from pithed frogs were kept in cold solution resembling amphibian ECF of the following composition (in mM): NaCl 117, KCl 3, Na_2HPO_4 0.8, NaH_2PO_4 0.2, $CaCl_2$ 1, $MgCl_2$ 1, glucose 10 at a pH of 7.4 ± 0.02. The atria of the isolated heart were completely removed and the apex of the ventricle was cut. The circular strip of ventricular tissue thus obtained was cut in between two ligatures and a vertical strip was obtained. One end of the strip was anchored with a silk thread to the base of a cylindrical bath and the other end was connected to an isometric force transducer. The force transducer was connected to a physiographic chart recorder.

Two silver stimulating electrodes were placed on either sides of the tissue such that they created a field potential. The bath with the tissue was superfused with oxygenated control solution at 23°C to 27°C. The tissue was regularly paced with an external stimulus at a frequency of 0.2 Hz and the force of the isometric contraction was recorded on a physiographic chart recorder.

Stimulus protocol

The tissue was allowed to stabilize in the control solution for at least 45 min. After a steady state of contraction was observed, the steady pacing was interrupted and varying rest periods ranging from 20 to 100 s and 180 s were imposed on the tissue in a random manner. The amplitude of the first post rest beat was noted. Each rest interval was given after ensuring that the force of contraction had reached the steady state after recovery from the previous rest period. Exhibited force of contraction was considered to be an index of the calcium availability for the contraction. The amplitude of the post rest beat is expressed as a percentage of that of the pre rest beat. If the amplitude of the post rest beat was smaller than the amplitude of the beat just prior to the rest interval, the phenomenon is referred to as RID. If the post rest amplitude was larger than the pre rest amplitude, then the phenomenon is referred to as RIP.

After the rest protocol was done in control conditions, the anti-arrhythmic agent was added to the perfusate and the same tissue was allowed to stabilize with the drug. After a steady state of contraction was achieved, similar rest protocol was employed with the drug of intervention. Resulting change in the amplitude of the post rest beat compared with that of the pre rest beat was noted and expressed as percentage. Each tissue preparation was subjected to test the effect of only one drug. Rest protocol was imposed only after the amplitude of the force of contraction had stabilized to a new steady level.

Statistical analysis of data

For analysis, the amplitude of the first post rest beat is expressed as a percentage of the amplitude of the

beat just prior to the rest period. Statistical significance of the RID with different rest periods was assessed by Paired *t*-test. The percentage of post rest amplitude in relation to the pre rest amplitude after the drug intervention as compared to control conditions in the same preparation was assessed for significance by two-way analysis of variance. $P < 0.05$ was considered significant.

RESULT

Amiodarone (7.5 µM) (*n* = 4)

Anti-arrhythmic agent belonging to Class III, with ability to lengthen the refractoriness – RID pattern persisted and did not show any significant increase in post rest amplitude when compared to the pre rest amplitude [Table 1].

Quinidine (10 µM) (*n* = 4)

Anti-arrhythmic agent belongs to Class Ia – RID pattern persisted and did not show any significant increase in post rest amplitude when compared to the pre rest amplitude [Table 2].

Bepridil (10 µM) (*n* = 4)

Calcium channel blocker belonging to Class IV anti-arrhythmic agent – RID pattern persisted and did not show any significant increase in post rest amplitude when compared to the pre rest amplitude [Table 3].

Lignocaine (10 µM): (*n* = 4)

Local anaesthetic agent, parenterally used for treating ventricular arrhythmias, belonging to Class Ib anti-arrhythmic agent, did not show any significant increase in post rest amplitude when compared with the pre rest amplitude [Table 4].

DISCUSSION

Cardiac depolarisations that deviate from the normally characterized rhythmic beat (After depolarisation) result in enhanced automaticity. Cardiac arrhythmogenesis has multifactorial etiological implications-ischemia, hypoxia, acid base imbalance, electrolyte disturbances, catecholamine exposure, autonomic influences, drug toxicity, overstretching of fibres, presence of scarred or otherwise diseased tissue-all accounting for disturbed impulse generation or conduction or both. An abnormal after depolarisation may be either an early after depolarization or a delayed after depolarization caused by transient inward depolarizing ionic current.

Table 1: Comparison of the percentage of decay in post rest force with that of pre rest force between control and 7.5 mM amiodarone (*n* = 4)

Rest period in seconds	Control (%)	Amiodarone (%)
20	87.58±10.91	85.54±15.05
40	81.60±14.24	72.89±20.02
60	68.62±17.11	65.08±18.90
80	59.93±19.09	61.25±20.94
100	62.31±16.10	54.21±19.22
180	41.55±13.11	45.61±20.42
SEd	12.27945	
CD (*P*<0.05)	24.90665	

Table 2: Comparison of the percentage of decay in post rest force with that of pre rest force between control and 10 mM quinidine (*n* = 4)

Rest period in seconds	Control (%)	Quinidine (%)
20	101.82±5.14	106.42±13.10
40	97.15±8.08	106.90±19.99
60	96.18±8.82	100.37±21.23
80	82.16±11.54	92.36±19.23
100	87.32±11.32	85.52±19.35
180	62.40±14.61	65.92±20.84
SEd	10.88748	
CD (*P*<0.05)	22.08329	

Table 3: Comparison of the percentage of decay in post rest force with that of pre rest force between control and bepridil 10 mM (*n* = 4)

Rest period in seconds	Control (%)	Bepridil (%)
20	101.49±5.39	99.10±11.41
40	93.77±4.47	93.61±20.08
60	90.72±7.01	90.39±24.00
80	81.25±3.62	79.80±31.67
100	76.70±7.86	73.35±24.38
180	53.13±6.07	57.80±35.86
SEd	12.32305	
CD (*P*<0.05)	24.99510	

Table 4: Comparison of the percentage of decay in post rest force with that of pre rest force between control and 10 mM lignocaine (*n* = 4)

Rest period in seconds	Control (%)	Lignocaine (%)
20	99.877±4.109	94.57±7.03
40	91.858±3.367	87.39±6.44
60	89.552±2.983	80.32±11.85
80	80.021±6.351	66.64±19.80
100	81.633±5.914	67.05±14.67
180	58.953±15.200	41.55±15.29
SEd	8.08125	
CD (*P*<0.05)	16.39136	

In its normal working mode, NCX produces a net positive influx, which is responsible for membrane depolarization. [8] Extrusion of calcium by the NCX occurs during the diastolic phase which in turn influences membrane depolarization.[9] However it has been described that during physiological conditions the direction of NCX varies with the phases of action potential being transiently inward during the initial phase of action potential and then reverse during the later phase.[10]

Altered calcium handling may result in electrophysiological readjustments as it affects the working direction of NCX and may cause contractile variability in the cardiac tissue. SR when overloaded with calcium could cause an abnormal leak of calcium which may in turn up regulate NCX. This possibly could result in generation of a spontaneous depolarizing inward current and thus could be arrhythmogenic.[11]

With the given understanding of development of arrhythmia in a cardiac tissue, targeting the calcium movement in and out of SR should be the future insight in designing anti-arrhythmic strategies. An electrochemical gradient or an event that could reverse the direction of NCX to an intracellular calcium acquiring mode could thus abolish the abnormal impulse generation. This reversal of direction of NCX results in efflux of 3 Na^+ in exchange for influx of 1 Ca^{++}. The acquired calcium enhances the SR as shown by the amplification of the post rest force of contraction producing a RIP.

Organic calcium channel blockers with anti-arrhythmic activity, diltiazem and verapamil have been shown to reverse the NCX as shown by producing an amplification of the post rest contraction force (RIP).[6] Similar RIP could not be demonstrated with the other calcium channel blocker nifedepine, which does not possess anti-arrhythmic property. Other interventions with ionic alterations that are capable of making the tissue acquire calcium and extrude sodium such as, low sodium or high calcium in the ECF have also produced a similar RIP pattern.[6]

Anti-arrhythmic agents belonging to different classes according to Vaughan Williams classification, with action on ion channels when tested for their role in reversing the direction of NCX, did not show any significant change in the existing decay pattern of contractile force with an imposed rest period as observed with the control solution. This includes a calcium channel blocker (Bepridil)also. Hence it is concluded that the molecular mechanism of action of the above said anti-arrhythmic agents does not interfere with the normal working direction of NCX on the sarcolemma of the cardiac myocyte. However, it is noteworthy that some of these anti-arrhythmic agents are pro arrhythmic as well.

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