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EFFECTS OF CALCIUM, STRONTIUM, AND BARIUM ON ISOLATED PHRENIC NERVE-DIAPHRAGM PREPARATION OF RAT AND THEIR INTERACTIONS WITH DILTIAZEM AND NIFEDIPINE

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Abstract : Calcium (Ca^{2+}), strontium (Sr^{2+}), and barium (Ba^{2+}) are expected to exert similar chemical and pharmacological effects. The effects of barium, strontium and calcium were studied on the contractions of rat phrenic nerve-diaphragm preparations, following electrical stimulation and their interactions with nifedipine (nif) and diltiazem (DZM) were also studied.

Low doses of strontium chloride ($SrCl_2$), barium chloride ($BaCl_2$) and calcium chloride ($CaCl_2$) were able to increase the force of contraction of the rat diaphragm when actively stimulated. Diltiazem inhibited the stimulant effects of Sr^{2+}, Ba^{2+}, and Ca^{2+}. On the other hand, nifedipine blocked the effects of Sr^{2+} and Ca^{2+} but potentiated the effects of Ba^{2+}. Strontium, barium, and calcium restored the contractility of the muscle following electrical stimulation when the tissue was in biological fluid absolutely depleted of calcium. These findings suggest that Sr^{2+} and Ba^{2+} may be able to substitute Ca^{2+} in the rat diaphragm for its contractions and nifedipine and diltiazem may follow different mechanisms of actions or channels in their blocking effects.

Key words : calcium strontium barium nifedipine diltiazem rat phrenic nerve-diaphragm preparation

INTRODUCTION

Barium (Ba^{2+}) and strontium (Sr^{2+}) being placed in the same group as that of calcium (Ca^{2+}) in the Periodic Table, are expected to exert similar chemical influence and thereby produce similar pharmacological effects. In a parallel study, a comparison was made between the potencies of external Ca^{2+}, Sr^{2+}, and Ba^{2+} to support GABA-ergic synaptic transmission in rat cultured hippocampal neuron. The results strongly suggested that Ba^{2+} as well as Sr^{2+} could be substituted for Ca^{2+} in GABA-ergic synaptic transmission and the order of potency was $Ca^{2+} > Sr^{2+} > Ba^{2+}$[1]. In a yet, another study it was shown that Ca^{2+} could be replaced in reconstitution process of the quinoprotein menthol dehydrogenase by Sr^{2+} or Ba^{2+}, the affinities for these ions being similar to that

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of Ca^{2+}[2]. In order to explore the possibility of similar effects by calcium, strontium, and barium on a mammalian skeletal muscle, the present study was undertaken to observe the effects of Ba^{2+}, Sr^{2+}, and Ca^{2+}, on the contraction of rat phrenic nerve-diaphragm preparation following electrical stimulation. The blockade of the action of Ba^{2+}, Sr^{2+}, and Ca^{2+} on this preparation may point to the underlying mechanism of these cations. Therefore, the effects of nifedipine (nif) and diltiazem (DZM) and their interactions with the above mentioned cations on the said tissue were also studied, in the anticipation that these cations may follow the same pattern and ion channels as that of calcium.

METHODS

Albino rats of wistar strain of either sex weighing 150–200 g with free access to standard diet and tap water were used. The rat was sacrificed and the diaphragm along with the phrenic nerve was dissected out and mounted in an isolated organ bath and was electrically stimulated with 1 volt to produce contractions of the muscle by the method described by Edith Bulbring in 1946[3] with some modifications. The Institution's guidelines for the care and use of laboratory animals were strictly followed.

The drugs used were strontium chloride ($SrCl_2$) [Analar, BDH], barium chloride ($BaCl_2$) [GR Sarabai Chemicals], calcium chloride ($CaCl_2$) [Analar, BDH], diltiazem [Torrent Pharmaceuticals Ltd, Ahmedabad] and nifedipine [Cadila Healthcare Pvt Ltd, Ahmedabad].

The electrically induced contractions were elicited in presence of barium chloride in the dose range of 1–8 μg/ml, strontium chloride in the dose range of 200–400 μg/ml and calcium chloride in the dose range of 80–100 μg/ml in Tyrode solution.

The contractions were then induced in presence of barium, strontium, and calcium in the respective dose ranges but pretreated with diltiazem (100 μg/ml) and then with nifedipine (0.8 μg/ml) 60 seconds before the addition of the respective cation in the 5-minute cycle of the contraction procedure. The contractions were also induced using Kreb's solution depleted of calcium but in presence of strontium or barium.

Statistical analysis was conducted using Student paired 't' test and found to be significant.

RESULTS

On the electrically induced contractions of phrenic nerve-diaphragm preparation, Ba^{2+} produced a slight potentiation (Table I) in the dose range of 1–8 μg/ml.

Similarly Sr^{2+} showed potentiation of the contraction in the dose range of 200–400 μg/ml (Table I) and Ca^{2+} also showed potentiation in the dose range of 80–100 μg/ml. Diltiazem inhibited the stimulant effects of Sr^{2+}, Ba^{2+}, and Ca^{2+} in the dose of 100 μg/ml whereas, nifedipine blocked the effects of Sr^{2+} and Ca^{2+} but potentiated the effect of Ba^{2+} in the dose of 0.8 μg/ml (Table I).

TABLE I: Effect of calcium, strontium and barium per se and in presence of diltiazem and nifedipine separately on the contractions of isolated rat-phrenic nerve-diaphragm preparation.

Response of isolated phrenic nerve-diaphragm preparation in amplitude (mm)				
Stages	*Groups*	*Before drug administration Mean±S.D.*	*After drug administration Mean±S.D.*	*% Change*
I	$BaCl_2$	36.51±11.87	46.5±13.61	27.39
II	$SrCl_2$	20.75±11.41	28.36±9.01	36.6
III	$CaCl_2$	31.25±12.14	38.26±13.01	22.43
IV	$BaCl_2$ + DZM	40.46±18.96	19.5±4.27	–52.7
V	$SrCl_2$ + DZM	37.46±13.05	6.11±2.69	–83.5
VI	$CaCl_2$ + DZM	35.51±11.06	12.17±1.13	–66.5
VII	$BaCl_2$ + nif	49.35±10.16	65.16±5.78	32.9
VIII	$SrCl_2$ + nif	30.48±8.27	12.51±2.09	–59.3
IX	$CaCl_2$ + nif	34.14±9.21	18.61±2.34	–55.27
X	$BaCl_2$ + $CaCl_2$	23.01±8.62	9.68±1.03	–58.0
XI	$SrCl_2$ + $CaCl_2$	26.41±9.8	16.61±2.31	–36.5
XII	$CaCl_2$ + $CaCl_2$	32.21±9.5	15.21±2.52	–52.8

(Footnote for Table I)
All the observations were statistically significant with P value <0.05.

The groups X, XI, and XII indicate the effects of barium chloride, strontium chloride, and calcium chloride in presence of calcium depleted Kreb's solution.

$BaCl_2$ – barium chloride; $SrCl_2$ – strontium chloride;
$CaCl_2$ – calcium chloride; DZM – diltiazem; nif – nifedipine

Sr^{2+}, Ba^{2+}, and Ca^{2+} partially restored the contractility of the muscle, following electrical stimulation when the tissue was depleted of calcium in the physiological solution (Table I).

In summary, barium and strontium potentiate the contractions of rat phrenic nerve-diaphragm preparation. However, in presence of diltiazem they are inhibited whereas the barium effect in presence of nifedipine was potentiated. Both barium and strontium recovered the contractions of the diaphragm in calcium-depleted solution.

DISCUSSION

The contractions of the diaphragm increased in presence of Sr^{2+}, Ba^{2+}, and Ca^{2+}. The possibility of an inward flow of divalent cations to play a role in the excitation-contraction coupling process, as first described by Bernard, et al (4) is further strengthened by the present finding.

Both barium and strontium in small doses have been shown to potentiate the contractions of the rat diaphragm following electrical stimulation. However, in higher doses, they inhibit the contractions. Charles Edwards et al (5) have shown that strontium fully activates the myofibrillar adenosine triphosphate. This may be taken as the possible explanation for the increased contractions of the diaphragm in small doses of strontium chloride. The same explanation may also hold true for barium chloride.

Moreover, Ca^{2+} is found to bind with highest affinity to calcium-gated cardiac muscle calcium release channel; and Ba^{2+} was found to compete with Ca^{2+} for calcium activation gates; possibly another explanation for the Ba^{2+} potentiation of contraction (6).

The inhibited response produced by strontium chloride in higher doses may be explained by the action of the cation on the membrane permeability that it stabilizes the muscle membrane leading to the inhibitory effect. Similarly, Premendran et al (7) and Silinsky (8) have reported that barium inhibits contractions by membrane stabilization.

When the rat diaphragm was bathed in partially calcium-depleted solution the contractions were very much reduced. However, addition of small amounts of barium chloride and strontium chloride could recover the height of contractions to certain extent, whereas in absolutely calcium-depleted solution, barium could never improve the contraction of the diaphragm. This is in agreement with the findings of Kawata (9) and Noguera (10) suggesting the release of calcium from the sarcoplasmic reticulum by barium ion during the contractile process of the skeletal muscle. Moreover, Sr^{2+} can pass through Ca^{2+} entry pathway activated by Ca^{2+} depletion (11).

Diltiazem and nifedipine have been shown to directly inhibit the contractions of the diaphragm. These drugs being calcium channel blockers, this action was anticipated. However, it has been shown that diltiazem also inhibits the contractions of diaphragm in presence of strontium and barium. This shows that diltiazem blocks the action of Sr^{2+} and Ba^{2+} in a similar fashion as that of calcium through a nonspecific channel system as also evidenced by Kohlhard et al (12) and Samolora (13).

On the other hand, nifedipine potentiated the contractions of the diaphragm in presence of barium and inhibited the contractions in presence of strontium. Roed (14) has stated that the stimulation due to the presence of nifedipine is by a delay of fatigue induced accumulation of K^+ in the T tubules which may occur during nifedipine induced reduction of $[Ca^{2+}]_i$ which in turn stimulated K^+ efflux leading to potentiation. In a study on rat skeletal muscle, dihydropyridine type calcium channel antagonist was shown to increase vascular permeability when injected locally, that is, it increases the permeability of post-capillary venule. To some extent it may explain the Ba^{2+} potentiated contractions in presence of a blocker (15).

However, it was shown that nifedipine blocked the effect of strontium chloride on rat diaphragm. Barium being a potassium channel blocker could not make a re-entry for K^+ ions caused by nifedipine in barium potentiation but with strontium, which is not a potassium channel blocker and hence there was a re-entry of K^+ which inhibited the strontium induced potentiation of rat diaphragm contractions. This is only a hypothesis and requires more confirmatory experiments to prove.

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