Mechanism of Action of the New Cardiotonic Suphan on Calcium Exchange in Cardiomyocytes

P. A. Galenko-Yaroshevskii, A. I. Khankoeva, and A. S. Dukhanin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 7, pp. 57-59, July, 1996
Original article submitted July 27, 1995

Effects of suphan, a new cardiotonic agent containing succinyl tryptophan, on the entry of Ca^{2+} into rat cardiomyocytes, its intracellular compartmentalization, and its exit from these cells were evaluated *in vitro*. It was found that the recorded sulfan-induced rise of intracellular calcium was due to Ca^{2+} entering the cell via L-type calcium channels, and that a reversible reduction of its concentration in the sarcoplasm occurred through its accumulation in the sarcoplasmic reticulum and was blocked by the specific Ca^{2+}-ATPase inhibitor thapsigargin (10 μM). Suphan did not alter the activity of Na^+/Ca^{2+} exchange in a concentration range of 5-150 μg/ml.

Key Words: *calcium; cardiomyocytes; suphan; Ca-ATPase*

The cardiotonic action of many drugs is based on their ability to activate calcium (Ca) exchange in myocardial cells. Ca^{2+} is directly involved in the coupling of myocardial electrical and mechanical activities, by virtue of which the force of cardiac contraction is directly proportional to the concentration of free Ca^{2+} in the sarcoplasm. A rise of Ca^{2+} in response to cardiotonics may be a consequence of both the entry of extracellular Ca^{2+} into cells via voltage- and/or receptor-controlled Ca channels [5] and the mobilization of Ca^{2+} from its intracellular depots, predominantly sarcoplasmic reticulum (SPR) cisterns [7].

Suphan, a new preparation proposed for use as a cardiotonic, contains succinyl tryptophan [3]. Studies into the impact of sodium succinate on the bioenergetics of ischemic myocardium have yielded a large body of experimental data, including those attesting to its membrane-stabilizing and hypoxia-mitigating effects [2]. However, there is only indirect evidence of how succinates and suphan might influence Ca exchange in the myocardium.

Department of Pharmacology, Kuban Medical Academy, Krasnodar; Department of Molecular Pharmacology with a Course in Radiobiology, Russian State Medical University, Moscow

The purpose of this study was to examine, using the Fura 2-AM fluorescent probe for calcium, the influence of suphan on intracellular Ca^{2+} concentration in isolated rat cardiomyocytes (CMC).

MATERIALS AND METHODS

The following reagents were used: $CdCl_2$ and $NiCl_2$ (Fluka); HEPES, NaCl, KCl, $CaCl_2$, NaH_2PO_4, $MgSO_4$, EGTA, and thapsigargin (Sigma); Fura 2-AM (Calbiochem); $MnCl_2$, digitonin, caffeine and epinephrine (Serva); and chemically pure HCl and NaOH (Russian-made).

The procedures used to isolate CMC from the left ventricle of rats, to load these cells with the Fura 2-AM probe, and to record its fluorescence are described in our previous article [4]. Intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) were calculated by the formula: $[Ca^{2+}]_i=K(R-R_{min})/(R_{max}-R)$, in which R_{min} and R_{max} are the ratios of fluorescence intensities at excitation wavelengths of 340 nm and 380 nm (F_{340}/F_{380}) at zero and saturating Ca^{2+} concentrations, respectively, and K is defined as $K_d\times(F_O/F_S)$, where F_S=fluorescence at 380 nm of the Ca^{2+}-free Fura 2-AM probe, F_O=fluorescence at 380 nm of the probe complexed with Ca^{2+} [1], and K_d=equilibrium dis-

0007-4888/96/0007-0701$15.00

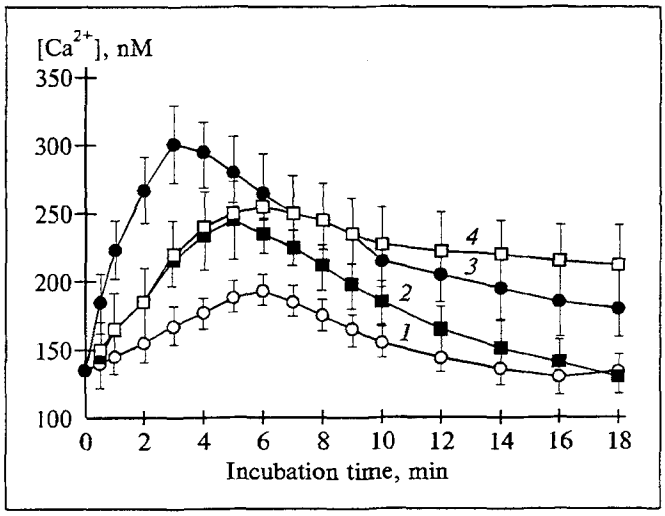

Fig. 1. Time-course of the Ca response by isolated rat cardiomyocytes exposed to suphan. The values given are means ± confidence intervals as calculated for 5-7 separate tests. Suphan concentrations (μg/ml): 20 (*1*), 50 (*2*), 100 (*3*); 50 μg/ml suphan+10 μM thapsigargin (*4*).

sociation constant for the probe-Ca^{2+} complex; this constant, which was determined in a model experiment using Fura 2-AM solution, equalled 140 nM when calculated by Scatchard analysis.

The results were statistically treated using the application software package Pharmacological Basic Statistics. Confidence intervals for experimental values and the significance of differences between them were estimated by Student's *t* test, setting statistical significance at the 0.05 level.

RESULTS

Suphan elicited no Ca response from CMC in concentrations below 5 μg/ml, but raised, in a dose-dependent manner, the basal (diastolic) Ca^{2+} level in these cells in a concentration range of 5-150 μg/ml. When incubated with CMC for 5 min in the concentration of 150 μg/ml, the recorded $[Ca^{2+}]_i$ was [301±28 nM (n=4) *vs.* 135±11 nM in the control samples (n=9)], i.e., 2.3 times higher. No significant change in $[Ca^{2+}]_i$ occurred at higher suphan concentrations in the incubation medium.

The time-course of the Ca response by CMC was biphasic (Fig. 1), with a rise in Ca^{2+} during the first 2-7 min after the addition of suphan to the incubation medium being succeeded by its fall in the subsequent 2-10 min. It should be noted that, as is illustrated in Fig. 1, the Ca^{2+} level declined to its initial value toward the end of the incubation period at suphan concentrations of ≤50 μg/ml, but not at higher concentrations.

Examination of the mechanisms underlying the suphan-induced elevation of $[Ca^{2+}]_i$ in phase I and its fall in phase II led us to conclude that the elevation was determined by the entry of Ca ions via the L type of sarcolemmal Ca channels. This conclusion is based on two pieces of evidence: 1) 60-70% decreases in the Ca response of CMC to suphan in calcium-free medium and 2) a virtually complete inhibition of this response by bivalent cadmium and nickel ions (0.2 mM $CdCl_2$ and 1 mm $NiCl_2$), which are selective blockers of L-type Ca channels (Fig. 2). The activation of Ca^{2+} entry by suphan appears to be due to its high content of potassium ions which may cause partial depolarization of CMC membranes and opening of potential-dependent Ca channels under *in vitro* conditions [3].

Systems maintaining $[Ca^{2+}]_i$ at a low level in CMC are (in order of decreasing functional significance) the SPR ATPases, the Na^+/Ca^{2+} antiport, and the Ca-ATPases located in the sarcolemma [6]. To determine what role the SPR might play regulating the suphan-induced rise in $[Ca^{2+}]_i$, we used the specific Ca-ATPase inhibitor thapsigargin (10 μM) [7]. In the presence of this compound, the kinetics of the Ca response in phase II was found to have changed (Fig. 1), so that the period of Ca^{2+} removal from the cytoplasm became 3 to 5 times longer than at the same suphan concentration in its absence.

In the presence of caffeine (10 mM), which depletes Ca^{2+} stores in the SPR and prevents Ca^{2+} reentry, the kinetics of suphan was monophasic. Amiloride (40 μM), an inhibitor of Na^+/Ca^{2+} transport, did not affect its kinetics.

The results presented above indicate that suphan stimulates the entry of extracellular Ca^{2+} into CMC where it accumulates in the SPR. Because of

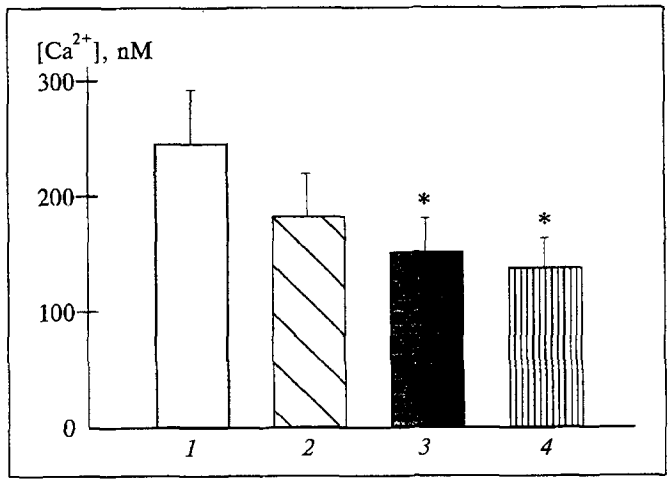

Fig. 2. Response of rats cardiomyocytes to suphan in different media. *1*) Control; *2*) calcium-free medium; *3*) medium containing 0.2 mM $CdCl_2$; *4*) medium containing 1 mM $NiCl_2$. *Significant difference from the control (p<0.05). $[Ca^{2+}]_i$ was measured 5 min after adding suphan at 50 μg/ml.

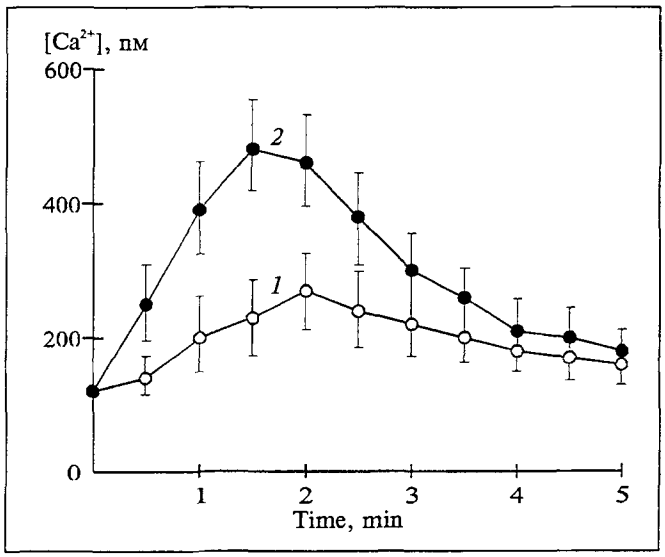

Fig. 3. Ca response of intact (*1*) and suphan-treated (50 μg/ml) (2) cardiomyocytes to epinephrine (10 nM).

the augmented Ca^{2+} accumulation in the SPR, subsequent activation of these cells may be expected to result in enhanced Ca release (an effect characteristic of cardiotonics stimulating β_1-adrenergic receptors). To check this possibility, we compared Ca^{2+} concentrations in control and suphan-treated (50 μg/ml) CMC. When the Ca^{2+} level stabilized (which happened after 20 min of observation), cells in the control and test samples were activated by adding 10 nM epinephrine, and its was found that the rate of rise in $[Ca^{2+}]_i$ and its maximal value in the test samples were 1.8-2.2 times than in the control ones (Fig. 3).

In summary, this study has shown that the impact of suphan on Ca homeostasis in CMC is determined by its ability to raise the diastolic level of Ca^{2+} and potentiate Ca^{2+} exit from the SPR in response to humoral (and possibly electrical) stimulation. These properties of suphan are consistent with its pharmacological actions on the heart *in vivo*.

REFERENCES

1. P. V. Avdonin and V. A. Tkachuk, in: *Receptors and Intracellular Calcium* [in Russian], Moscow (1994), pp. 36-39.
2. V. V. Gatsura, in: *Pharmacological Correction of Energy Metabolism in an Ischemic Myocardium* [in Russian], Moscow (1993), pp. 82-95.
3. A. I. Grinevich, I. S. Chekman, P. A. Galenko-Yaroshevskii, *et al.*, *Dokl. Akad. Nauk Ukrainy*, No. 5, 169-173 (1994).
4. P. V. Sergeev, P. A. Galenko-Yaroshevskii, A. I. Khankoeva, and A. S. Dukhanin, *Byull. Eksp. Biol. Med.*, **121**, No. 3, 288-291 (1996).
5. Z. Ming, C. Nordin, and R. S. Aronson, *J. Cardiovasc. Electrophysiol.*, **5**, 323-334 (1994).
6. N. Negretti, S. C. O'Neill, and D. A. Eisner, *Cardiovasc. Res.*, **27**, No. 10, 1826-1830 (1993).
7. N. Negretti, S. C. O'Neill, and D. A. Eisner, *J. Physiol. (London)*, **468**, 35-52 (1993).