PHARMACOLOGY AND TOXICOLOGY

Effects of Isoproterenol and Suphan on the Concentration of Free Calcium Ions in Isolated Cardiomyocytes: a Comparative Study

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Isoproterenol and suphan, two cardioactive drugs with different mechanisms of action, are studied *in vitro* for their effects on calcium homeostasis in myocardial cells. Isoproterenol lowers the basal Ca^{2+} level in resting cardiomyocytes and potentiates its rise in these cells after their induction. Suphan stimulates reversible elevation of the diastolic Ca^{2+} concentration, causing increased calcium accumulation in the sarcoplasmic reticulum of cardiomyocytes. In an *in vitro* model of hypoxia, the Ca response to isoproterenol is significantly reduced, whereas that to dibutyryl cAMP is retained. The effect of suphan on the Ca^{2+} content of cardiomyocytes exposed to "chemical" hypoxia is 30-50% higher than its effect on the Ca^{2+} content of intact cells.

Key Words: *isoproterenol; suphan; calcium; cAMP; dibutyryl cAMP; hypoxia*

Isoproterenol (IP) and the new drug suphan (potassium salt of succinyl tryptophan) are cardiotonic agents activating Ca exchange in myocardial cells [1,2]. The mechanism of action of IP (isadrine, isoprenaline) involves interaction of this substance with β-adrenergic receptors, activation of the membrane-bound enzyme adenylate cyclase, and the consequent formation of a cyclic nucleotide, adenosine 2':3' cyclic monophosphate (cAMP). Owing to its physicochemical properties, IP is incapable of crossing the plasma membrane of target cells, and its action is mediated by an intracellular second messenger. Unlike IP, suphan penetrates through the sarcolemma to be hydrolyzed into its components which become directly involved in metabolic processes and stimulate energy metabolism in cardiomyocytes (CMC) [1].

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The transmembrane signal transduction and energy production in the cytoplasm and mitochondria undergo substantial modifications in various pathological conditions of the myocardium, such as ischemia, infarction, and cardiomyopathy. A direct relationship between intracellular Ca^{2+} exchange and the pathogenesis of several cardiovascular diseases, including essential hypertension, has been demonstrated [5].

The aim of the present study was to compare the effects of IP and suphan on the concentration of free Ca^{2+} ions in the cytoplasm ($[Ca^{2+}]_{cyt}$) of resting CMC and of those exposed to hypoxia *in vitro*.

MATERIALS AND METHODS

Isolated CMC were obtained from the left ventricle of rats as previously described [9]. The cells were perfused with a modified Krebs solution equilibrated with 95% O_2 and 5% CO_2 (pH 7.4). The isolation medium contained (mM): 142 NaCl, 1 $MgSO_4$, 5 KCl,

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1.5 NaH_2PO_4, and 5 glucose. After the cells were finally resuspended, 2.5 mM $CaCl_2$ was added. The Fura 2-AM fluorescent probe for calcium was then added to the CMC suspension (10-15 million cells/ml) to a final concentration of 5 μM, and the suspension was incubated with the probe for 20 min at room temperature. The cells were then sedimented by centrifugation at 200*g* to remove the nonincorporated probe. The procedure used to record fluorescence and calculate the intracellular Ca^{2+} concentration is described elsewhere [3].

Hypoxic conditions were simulated by incubating cells for 15 min at 37°C in a medium containing 1 mM KCN and 20 mM 2-deoxyglucose [8].

Working IP and suphan concentrations were selected so as to correspond to the median effective dose (ED_{50}) in *in vivo* tests: a dose level at which the pharmacological effect of IP or suphan is half of the maximal [4]. The final IP and suphan concentrations in the incubation medium were 30 nM and 25 μg/ml, respectively.

The results were statistically analyzed using the application package Pharmacological Basic Statistics.

RESULTS

Analysis of the effects produced by IP (30 nM) and suphan (25 μg/ml) on basal Ca^{2+} levels in intact CMC indicates that these two substances regulate Ca homeostasis in different ways. IP lowered $[Ca^{2+}]_{cyt}$ in resting cells: by minute 5 of incubation, the diastolic Ca^{2+} concentration was 55 mM lower than the initial level (150±13 nM) and remained unchanged at least during 20 min of the observation period. The effect of suphan was biphasic (we described its mechanisms earlier [1]). Thus, as shown in Fig. 1, the observed rise of Ca^{2+} is followed by a fall to the initial level by minute 15 of incubation. Under the action of IP and suphan, the cytoplasmic free Ca^{2+} is pumped into the sarcoplasmic reticulum (SPR) and deposited there.

Figure 2 shows alterations in $[Ca^{2+}]_{cyt}$ upon subsequent induction of CMC (replacement of extracellular Na^+ by an equimolar amount of K^+ [3]). In IP- or suphan-treated cells, the rise in $[Ca^{2+}]_{cyt}$ is significantly higher than in the control samples because of a rise in the Ca^{2+} accumulated in the SPR of CMC and released into the sarcoplasm under the action of IP or suphan. The effect from IP is more pronounced, the increase in Ca^{2+} over control values (ΔCa) being 210 nM on average *vs.* 90 nM with suphan. Ca^{2+} is released from the SPR via specialized calcium channels which are activated when the Ca^{2+} concentration rises in the adjacent cytoplasmic area and which are involved in propagating the "calcium wave." Exogenous

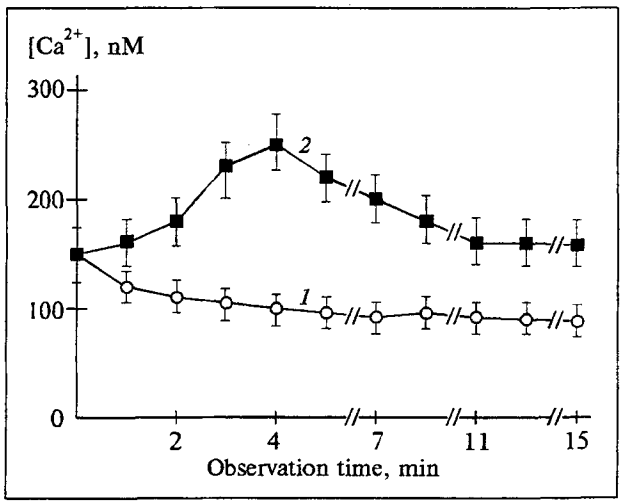

Fig. 1. Effects of isoproterenol (30 nM, *1*) and suphan (25 μg/ml, *2*) on the Ca^{2+} content of resting intact cardiomyocytes.

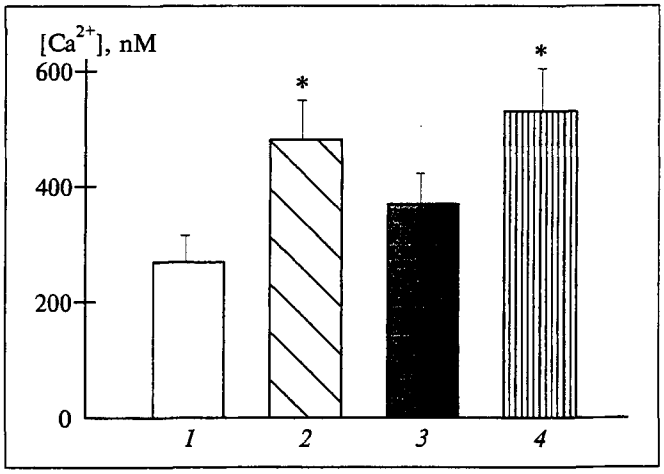

Fig. 2. Ca response to K^+ depolarization of intact cells (*1*) and cells exposed for 20 min to isoproterenol (*2*), suphan (*3*), and dibutyryl cAMP (100 μM, *4*). Here and in Fig. 4: final KCl concentration in the incubation medium was 40 mM and $[Ca^{2+}]_{cyt}$ was measured 3 min after cell induction. *Significant difference from control values ($p<0.05$).

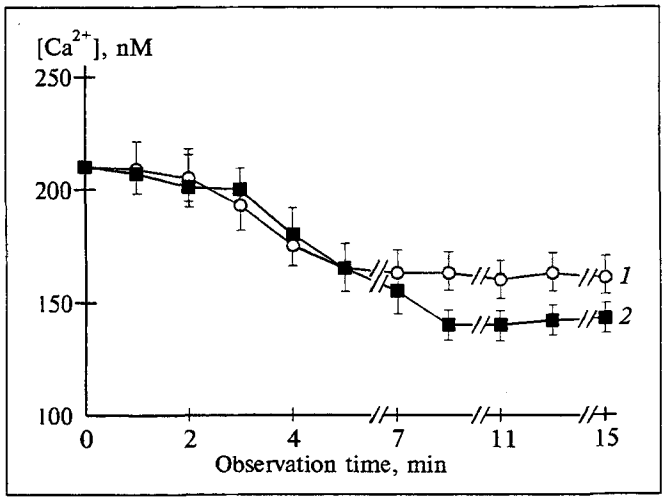

Fig. 3. Effects of isoproterenol (*1*) and suphan (*2*) on basal Ca^{2+} levels in CMC exposed to hypoxia.

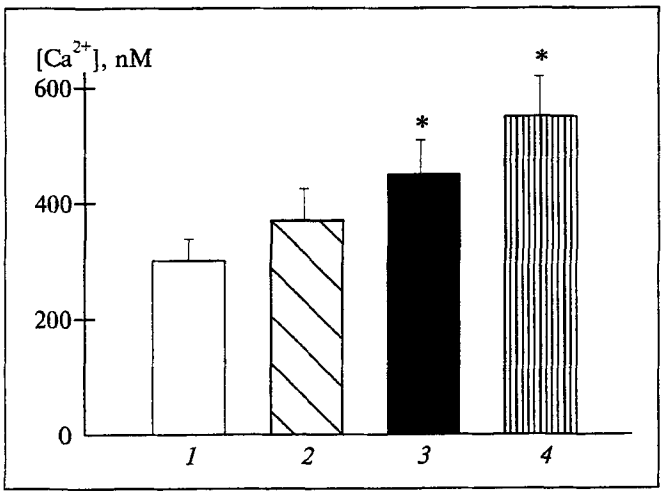

Fig. 4. Cell response to K^+ depolarization in the absence (*1*) and presence of isoproterenol (*2*), suphan (*3*), and dibutyryl cAMP (*4*) during "chemical" hypoxia.

activators of these Ca channels are caffeine and ryanodine which are effective in millimolar concentrations. That the source of $[Ca^{2+}]_{cyt}$ increase is the SPR is indicated by the ability of thapsigargin, a selective inhibitor of the SPR Ca-ATPase, to block the Ca-mobilizing action of IP and suphan [6,7].

In the second part of our study, the experimental scheme was the same as in the first except that CMC were exposed to "chemical" hypoxia. The estimated basal level of Ca^{2+} was higher than in intact cells (ΔCa=62 nM). This finding agrees with the observation by other authors that the activity of intracellular systems sustaining the diastolic $[Ca^{2+}]_{cyt}$ is reduced in myocardial hypoxia [10]. One of the factors responsible for Ca^{2+} rise is a decrease in the intracellular content of high-energy compounds, primarily ATP. When IP or suphan was added to the cell suspension, a fall in $[Ca^{2+}]_{cyt}$ was recorded (Fig. 3). It should be noted that, in this case, there was only one phase in suphan action: unlike in tests with intact CMC (Fig. 1), no initial rise in Ca^{2+} concentration was observed. After 15 min of incubation with IP and suphan, the $[Ca^{2+}]_{cyt}$ was 161±9 nM and 138±11 nM, respectively. Examination of the Ca response of CMC following their induction (Fig. 4) revealed an interesting phenomenon, namely, that suphan altered both the basal and stimulated $[Ca^{2+}]_{cyt}$ to a greater extent than did IP under hypoxic conditions, whereas in tests with intact cells IP was more active than suphan. To explain this phenomenon, we compared the sensitivity of CMC to IP with their sensitivity to dibutyryl cAMP. The dibutyryl derivative of cAMP has a spectrum of biological activity similar to that of cAMP but, unlike the natural cyclic nucleotide, readily passes across the cell membrane.

The effect of dibutyryl cAMP (100 μM) on $[Ca^{2+}]_{cyt}$ was observed in both cases (Figs. 2 and 4). The finding that CMC responded well to dibutyryl cAMP under hypoxic conditions may be taken as an indication that the sensitivity of these cells to IP is already impaired at the level of their plasma membrane, when the second messenger (cAMP) is formed. The increase in the relative biological effectiveness of suphan appears to be determined by its correcting influence on energy metabolism in myocardial cells.

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