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Induction of skeletal muscle contracture and calcium release from isolated sarcoplasmic reticulum vesicles by sanguinarine

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1 The benzophenanthrine alkaloid, sanguinarine, was studied for its effects on isolated mouse phrenic-nerve diaphragm preparations. Sanguinarine induced direct, dose-dependent effects on muscle contractility.

2 Sanguinarine-induced contracture was partially inhibited when the extracellular Ca^{2+} was removed or when the diaphragm was pretreated with nifedipine. Depletion of sarcoplasmic reticulum (SR) internal calcium stores completely blocked the contracture.

3 Sanguinarine induced Ca^{2+} release from the actively loaded SR vesicles was blocked by ruthenium red and dithiothreitol (DTT), consistent with the ryanodine receptor (RyR) as the site of sanguinarine action.

4 Sanguinarine altered [^{3}H]-ryanodine binding to the RyR of isolated SR vesicles, potentiating [^{3}H]-ryanodine binding at lower concentrations and inhibiting binding at higher concentrations. All of these effects were reversed by DTT, suggesting that sanguinarine-induced Ca^{2+} release from SR occurs through oxidation of critical SH groups of the RyR SR calcium release channel.
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Abbreviations: ACh, acetylcholine; APIII, antipyrylazo III; α-BuTx, α-bungarotoxin; DTT, dithiothreitol; MOPS, 3-(N-morpholino) propane sulphonic acid; RR, ruthenium red; SH, sulphydryl; SR, sarcoplasmic reticulum; TTX, tetrodotoxin.

Introduction

Sanguinarine is a benzophenanthridine alkaloid predominantly found in Papaveraceae, such as the roots of the blood root plant *Sanguinaria canadensis* L. (Mahady & Beecher, 1994), *Chelidonium majus* L. (Vavrecková *et al*., 1996) and the seeds of the *Argemone mexicana* L. (Tandon *et al*., 1975). Extracts of *Sanguinaria canadensis* have been shown to possess antioxidative (Firatli *et al*., 1994), antitumor, antibacterial activities, and antiinflammatory (Lenfeld *et al*., 1981) properties in animals, and to reduce gingival inflammation and supragingival plaque (Parsons *et al*., 1987; Laster & Lobene, 1990; Godowski *et al*., 1995) when used clinically. The Sanguinaria extract, sanguinarine, has been used in many over-the-counter products including toothpaste, mouthwash, cough and cold remedies, and homeopathic preparations (Frankos *et al*., 1990). Sanguinarine has a broad *in vitro* activity against gram-positive and gram-negative bacteria, fungi, and some protozoa (Nandi *et al*., 1983; Becci *et al*., 1987). It inhibits several enzymatic activities, including lipoxygenase (Vavrecková *et al*., 1996), cholinesterase, (Ulrichova *et al*., 1983a,b; Schmeller *et al*., 1997), and Na^+/K^+-ATPase (Seifen *et al*., 1979). It is also a potent inhibitor of tubulin assembly (Wolff & Kniping, 1993), NF-κB activation, IκBα phosphorylation, and degradation (Chaturvedi *et al*., 1997) and human polymorphonuclear cell chemotaxis, chemokinesis, and adherence (Agarwal *et al*., 1991).

Sanguinarine has been implicated in outbreaks of human poisoning known as epidemic dropsy which is characterized by oedema of the legs, congestive heart failure, hepatomegaly, ataxia, and glaucoma (Dalvi, 1985). Sanguinarine is toxic to vertebrates when injected intraperitoneally or fed by mouth (Sarkar, 1948) or when administered intravenously (Schmeller *et al*., 1997). The acute oral LD_{50} in rats was calculated to be 1658 mg Kg^{-1} with death resulting mainly from respiratory paralysis (Becci *et al*., 1987); however, the underlying mechanism leading to respiratory failure is not clear.

In this study, the direct effects of sanguinarine on isolated phrenic-nerve diaphragm preparations were investigated. Data are presented which suggest that sanguinarine induces profound muscle contracture through direct actions on the sarcoplasmic reticulum calcium release channel.

Methods

Chemical

All reagents were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) unless specified.

Mouse diaphragm preparation

Mice (ICR strain) weighing 20–25 g of either sex, purchased from Animal Center of College of Medicine, National Taiwan University (Taipei, Taiwan), were used for all experiments. The diaphragm was isolated according to the method of Bülbring (1946) and placed in Krebs solution of the following composition (mM): NaCl 118.5, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 2.5, glucose 11.1 and $CaCl_2$ 2.5; pH 7.4 and constantly gassed with 95% O_2 + 5% CO_2 at 37 ± 0.5°C in a 10-

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ml organ bath. For Ca^{2+} free experiments, the diaphragm preparations were first washed three times in Krebs solution with $CaCl_2$ replaced by 2.5 mM EGTA, and then placed in Ca^{2+} free Krebs solution without added $CaCl_2$ and EGTA. Twitches of the diaphragm were elicited by either stimulation of the phrenic nerve (indirect stimulation) with a supramaximal rectangular pulse of 0.05-msec duration at 0.2 Hz or muscle (direct stimulation) with a pulse 0.5-msec duration at 0.1 Hz in the absence or in the presence of $1 \times 10^{-6} \times g$ ml^{-1} of α-bungarotoxin (α-BuTx) or 1 μM tetrodotoxin (TTX). The muscle resting tension was set at 1 g, and the changes of tension were recorded *via* isometric transducer (Grass FT.03) on a Gould RS3200 polygraph (Gould Instrument Co.)

Preparation of sarcoplasmic reticulum fraction

The triad enriched heavy fraction of SR was prepared from back muscles of either rat or rabbit by a differential centrifugation as previously described (Kang *et al.*, 1994). Briefly, the muscles were homogenized and centrifuged at $10{,}000 \times g$ for 3 min in a JA-14 rotor (Beckman) and the supernatant fraction filtered through eight layers of cheesecloth and then centrifuged at $17{,}000 \times g$ for 60 min. The sedimented fraction was homogenized in a solution containing 0.3 M sucrose, 150 mM KCl, 0.2 mM phenyl methyl sulphonyl fluoride, and 20 mM 3-(N-morpholino) propane sulphonic acid (MOPS) (pH 6.8), and centrifuged at $17{,}000 \times g$ for 50 min in a JA-20 rotor (Beckman).

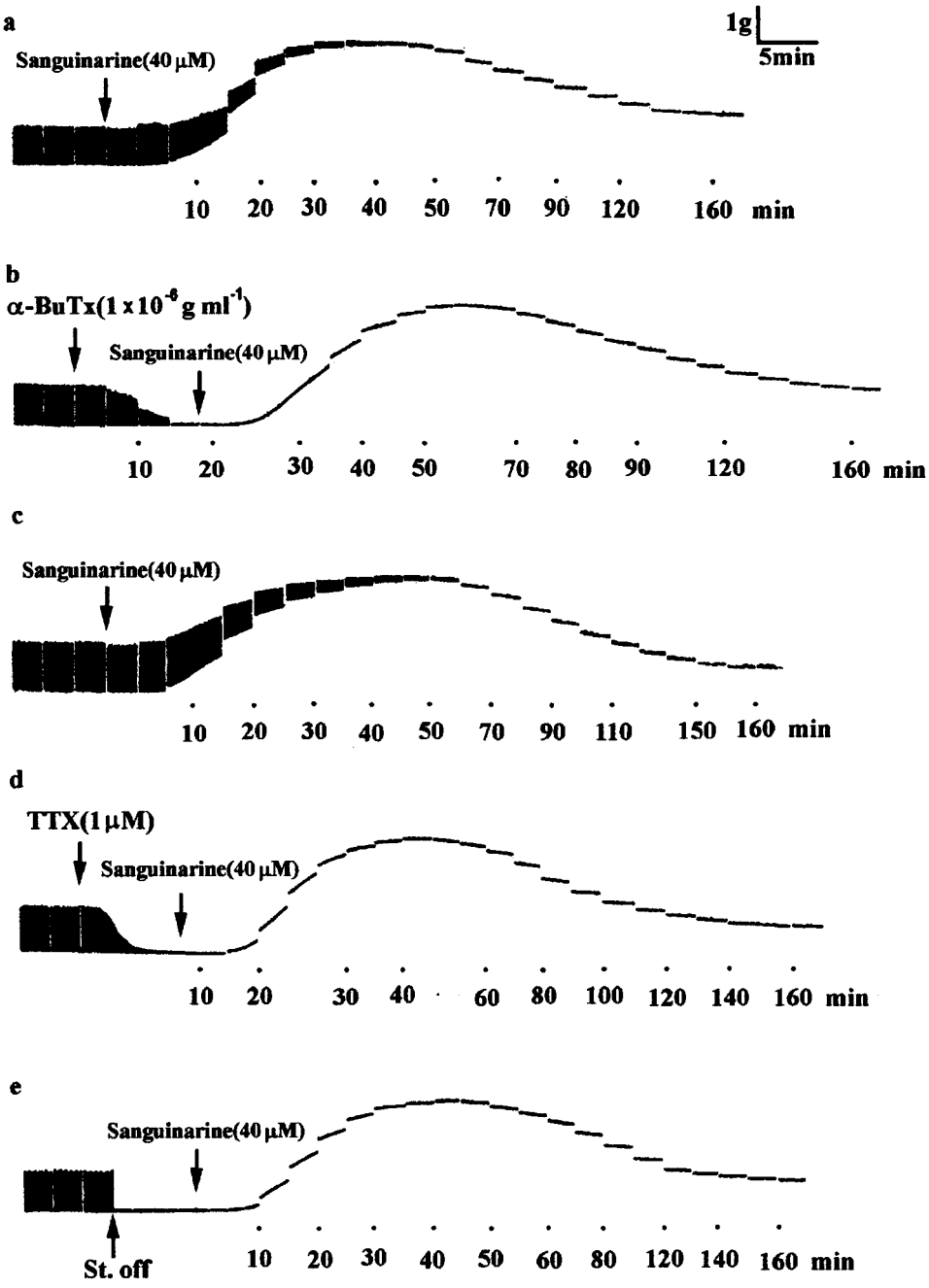

Figure 1 Sanguinarine-induced muscular contracture and paralysis of mouse diaphragm. The effects of 40 μM sanguinarine on nerve-stimulated (trace a and b) or muscle-stimulated (trace c and d) or quiescent (trace e) phrenic nerve-diaphragm isolated from mouse were studied according to the procedure outlined in Methods. In trace b and d, 1×10^{-6} g ml^{-1} α-bungarotoxin (α-BuTx) and 1 μM tetrodotoxin (TTX) were added prior to the addition of sanguinarine, respectively.

The final sedimentable fraction was homogenized in the above solution at a final protein concentration of 20–30 mg ml^{-1}. The preparation was quickly frozen in liquid nitrogen after protein determination and stored at −70°C.

Calcium release assay

The time course of Ca^{2+} release from SR vesicles was investigated with the Ca^{2+} sensitive probe, antipyrylazo III (AP III), in a dual wavelength spectrophotometer (SLM, Aminco DW-2000) modified from Palade (1987). SR vesicles (0.5 mg ml^{-1}), were actively loaded with the addition of 1 mM Mg-ATP in a reaction mixture containing 150 mM KCl, 100 μM AP III, 20 mM MOPS (pH 6.8). Additional aliquots of $CaCl_2$ were added sequentially until no more Ca^{2+} could be taken up into the SR vesicles. Release inducers, such as polylysine (2 μg ml^{-1}, M.W. 3800 Da) or sanguinarine at the concentration indicated, were added to induce Ca^{2+} release from SR.

Ca^{2+}-ATPase measurement

ATPase activity was determined with a coupled-enzyme spectrophotometric ADP-release assay (Warren *et al*., 1974) by measuring the oxidation of NADH at 340 nm with a Beckman DU-650 spectrophotometer. SR protein (20 μg) in the absence or presence of sanguinarine at different concentration was incubated in a 1 ml assay mixture buffer containing 20 mM MOPS (pH 6.8), 0.3 mg ml^{-1} NADH. 5 mM $MgCl_2$, 0.2 mM EGTA, 0.45 mM phosphoenolpyruvate, 5 units ml^{-1} pyruvate kinase, 10 units ml^{-1} lactate dehydrogenase and 4 μM of the Ca^{2+} ionophore A23187, at 37°C for 5 min, and the reaction was started by the addition of 100 μM ATP. Ca^{2+}-ATPase activity was calculated as the difference of activities measured with and without the addition of 0.2 mM $CaCl_2$.

[^{3}H]-ryanodine binding

Ryanodine binding was measured according to Pessah *et al.* (1987) with slight modifications. SR, 0.5 mg ml^{-1}, was incubated at 37°C for 2 h in a medium containing 250 mM KCl, 15 mM NaCl, 50 mM $CaCl_2$, 10 nM [^{3}H]-ryanodine (68.3 Ci $mmol^{-1}$; NEM, U.S.A.), 20 mM Tris, pH 7.1 in the absence or presence of sanguinarine at the concentrations indicated in each experiment. Non-specific binding was measured in the presence of 1 μM cold ryanodine (Latoxan, France). At the end of the incubation, 900 μl of each reaction mixture was withdrawn and added to 5 ml ice-cold buffer to quench the reaction, rapidly filtered through Whatman GF/B glass fibre filters, and rinsed once with 5 ml ice-cold buffer. The data shown are the average of triplicate determinations in at least three different preparations.

Statistical analysis

The statistical significance of difference between control and drug effects was evaluated by Student's *t*-test. A P value of 0.05 or less was considered statistically significant.

Results

Sanguinarine effects on contractility

The effect of sanguinarine on skeletal muscle was investigated by using preparations of phrenic-nerve diaphragm isolated from mice. As shown in Figure 1, sanguinarine induced a marked contracture in preparations which were either nerve-stimulated (trace a and b), activated by direct muscle stimulation (trace c and d) or in quiescent mouse diaphragms (trace e). The maximal contracture forces induced by sanguinarine were 3.18±0.15 g with nerve-stimulation, 3.21±0.16 g with direct muscle activation. Sanguinarine-induced contractures were not affected by α-BuTx (2.96±0.06 g, trace b) or TTX (3.15±0.16 g, trace d) treatments. These data suggested that the contracture induced by sanguinarine might be myogenic and independent of innervation.

Importantly, the sanguinarine-induced contractures were dose-dependent with maximum contracture force occurring at 50 μM sanguinarine (Figure 2a). Further, sanguinarine potentiated individual twitch contractions by decreasing the time to peak tension in a dose-dependent fashion (Figure 2b).

Previous studies have shown that the effect of sanguinarine on enzymatic activities were probably caused by its ability to react with the essential sulphydryl (SH) groups (Sarkar, 1948).

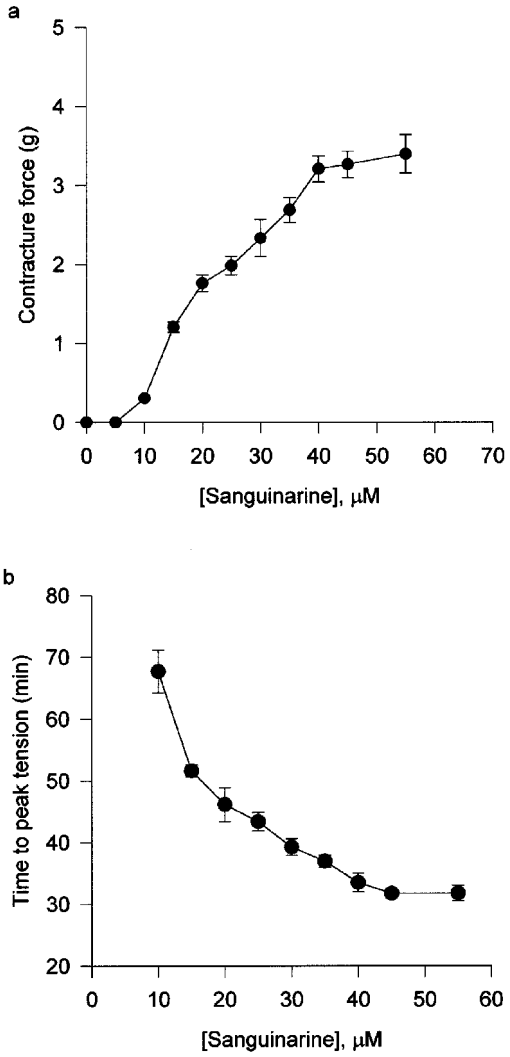

Figure 2 Dose-response effects for sanguinarine on muscular contracture in mouse diaphragm. Mouse diaphragm was prepared as described in Methods and the effects of sanguinarine at different doses on contractile force (a) and time to peak tension (b) were measured. Data are expressed as mean of multiple measurements and vertical lines show s.e.mean ($n>4$).

Table 1 Factors affecting sanguinarine-induced muscle contracture

	Contractile force (g)
Normal Krebs	3.21 ± 0.16
+ Nifedipine	2.48 ± 0.13^{a}
Ca^{2+}-free medium	2.29 ± 0.20^{a}
+ Nifedipine	2.24 ± 0.20^{a}
Ryanodine-pretreated	1.05 ± 0.18^{a}
+ wash with Ca^{2+} free medium	$0.32 \pm 0.16^{a,b}$

The contracture of isolated diaphragm was induced by 40 μM sanguinarine in various conditions, and tension measured according to procedure outlined in Methods. Nifedipine, at 1 μM, was added and incubated with the diaphragm for 10 min prior the addition of sanguinarine. For depletion of the internal Ca^{2+} pool, the diaphragm was pretreated with 2 μM ryanodine in normal Krebs medium and sanguinarine was added after the tension returned to base line. Data are expressed as mean ± s.e.mean ($n=4$). $^{a}P<0.01$, compared to normal Krebs medium; $^{b}P<0.01$, compared to ryanodine pretreated condition.

Thus, we examined the effects of the thio-reducing agent, dithiothreitol (DTT), on sanguinarine-induced contractures (Figure 3). Figure 3 shows that sanguinarine contractures could be rapidly reversed by the addition of DTT at the peak (trace b) and the beginning (trace c) of tension development. The saguinarine effect could be completely blocked with DTT pre-treatment (trace d).

Sanguinarine induced muscle contracture by induction of Ca^{2+} release from an internal Ca^{2+} storage site and enhanced the influx of extracellular Ca^{2+}

The involvement of extracellular Ca^{2+} and internal Ca^{2+} in sanguinarine-induced contractures were investigated by removal of extracellular Ca^{2+} with EGTA and depletion of internal Ca^{2+} store with ryanodine, respectively. The data are summarized in Table 1. The contractile force induced by sanguinarine was reduced by approximately 30% in Ca^{2+} free Krebs solution. Pretreatment with the L-type Ca^{2+} channel

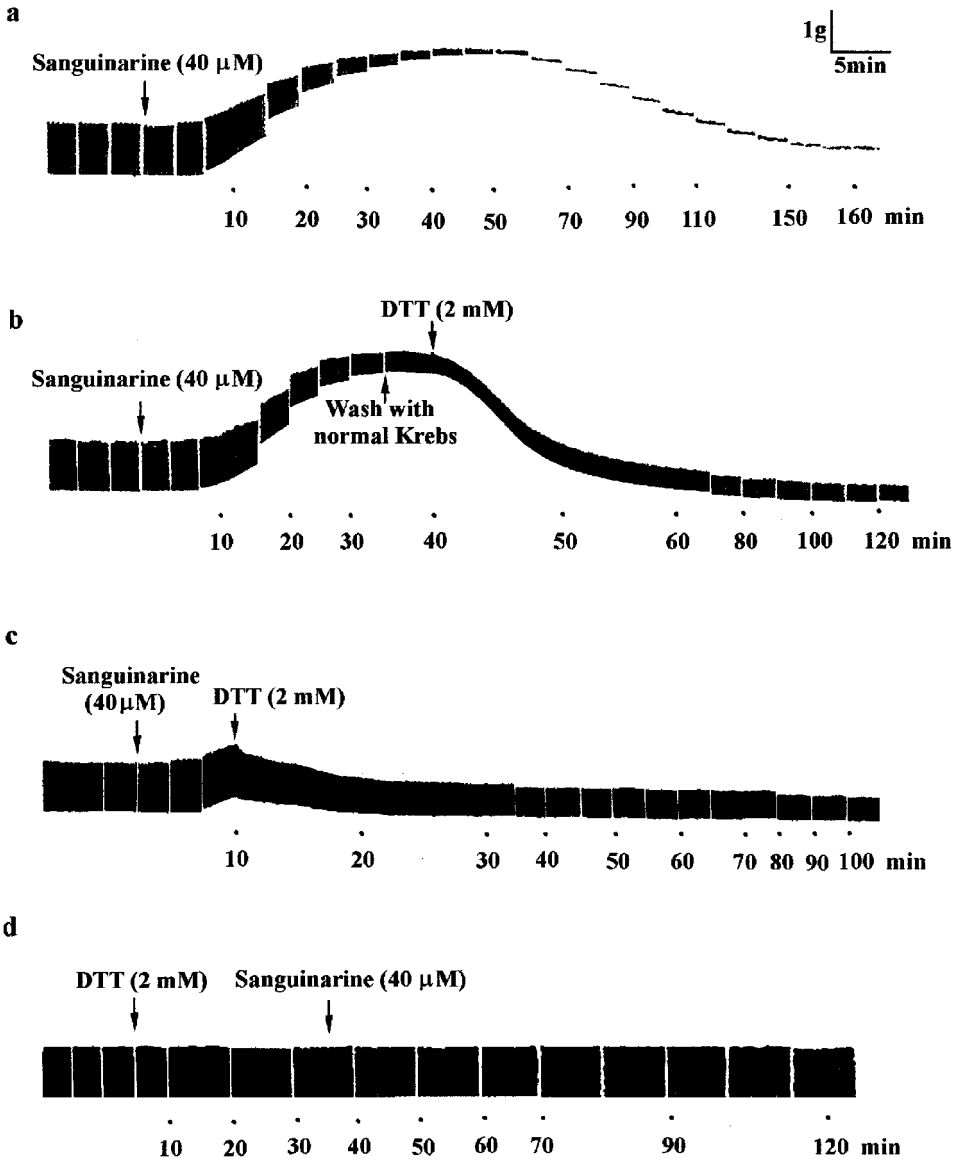

Figure 3 Reversal of the sanguinarine-induced contracture by sulphydryl reducing agent. The effect of sulphydryl reducing agent, dithiothreitol (DTT), on sanguinarine-induced contracture was examined. In trace b, the DTT was added after removal of the sanguinarine by washing the diaphragm with the fresh Krebs medium when the maximal contractile force was reached. In trace c, 2 mM of DTT was added at the beginning of the contracture without washing. In trace d, 2 mM of DTT was added prior to the addition of sanguinarine.

blocker, nifedipine, inhibited the sanguinarine-induced contracture in normal Krebs solution by approximately 25% but not in Ca^{2+} free solution. These data suggested that the contractures induced by sanguinarine were partly due to influx of extracellular Ca^{2+} through the L-type Ca^{2+} channel.

Significantly, sanguinarine-induced contractures were greatly reduced when the diaphragms were pretreated with ryanodine, used in concentrations which open the ryanodine receptor calcium release channel and deplete the SR of its calcium store. In these experiments, extracellular Ca^{2+} was chelated with EGTA. These observations suggested that SR internal calcium stores played an important role in the contracture induced by sanguinarine.

Sanguinarine induced Ca^{2+} release from sarcoplasmic reticulum

Involvement of the SR in sanguinarine effects was further studied in SR membrane preparations isolated from rabbit muscle (see Methods). The direct effects of sanguinarine on SR Ca^{2+} release were studied using the metallochromic Ca^{2+} indicator dye, antipyrylazo III, and a typical result was shown in Figure 4. Polylysine, a known release agent (Cifuentes *et al*., 1989), induced a rapid Ca^{2+} release from the SR (trace 1) as indicated by the sharp increase of optical absorbance difference at 710 nm and 790 nm. Sanguinarine, at 20 μM (trace 2) and 10 μM (trace 3), induced Ca^{2+} release from SR which could be blocked by prior addition of the specific Ca^{2+} release channel blocker, ruthenium red (RR, trace 4), or by the sulphydryl reagent, DTT (trace 5).

Effect of sanguinarine on [^{3}H]-ryanodine binding

Ryanodine, a plant alkaloid, binds specifically to the Ca^{2+} release channel of SR (Fleischer *et al*., 1985). Ligand binding was used as a probe for the channel activity (Chu *et al*., 1990). Figure 5 demonstrates that sanguinarine exerted a biphasic effect on [^{3}H]-ryanodine binding to SR membrane vesicles. At lower concentration (<20 μM), the drug potentiated [^{3}H]-ryanodine binding. However, the degree of potentiation decreased as the concentrations of sanguinarine increased to higher doses (20–40 μM). Binding was completely inhibited at 40 μM sanguinarine. The effects of sanguinarine, either at low or high concentrations, could be reversed by co-treatment with DTT (Table 2).

Discussion

In this study, we have presented evidence demonstrating that sanguinarine induces dose-dependent contractures of isolated

Table 2 Effect of sanguinarine on [^{3}H]-ryanodine binding to isolated SR vehicles

Additions	μM	*[^{3}H]-ryanodine binding* (c.p.m.)
DMSO		18,526 ± 1,129
DMSO + DTT		15,491 ± 1,454
Sanguinarine	6	28,549 ± 789[a]
	18	38,652 ± 2,954[a]
	60	549 ± 207[a]
Sanguinarine + DTT	6	16,293 ± 101
	18	17,167 ± 149
	60	17,692 ± 760

The ryanodine binding was measured according to the procedure stated in Methods in the presence of 6, 18, 60 μM of sanguinarine with or without the addition of 180 μM dithiothreitol (DTT). Control binding was measured with the addition of 0.2% solvent, DMSO, instead of sanguinarine. The specific binding in the presence of sanguinarine was compared to the control (DMSO) and analysed by Student's *t*-test. [a]$P<0.01$ ($n>3$).

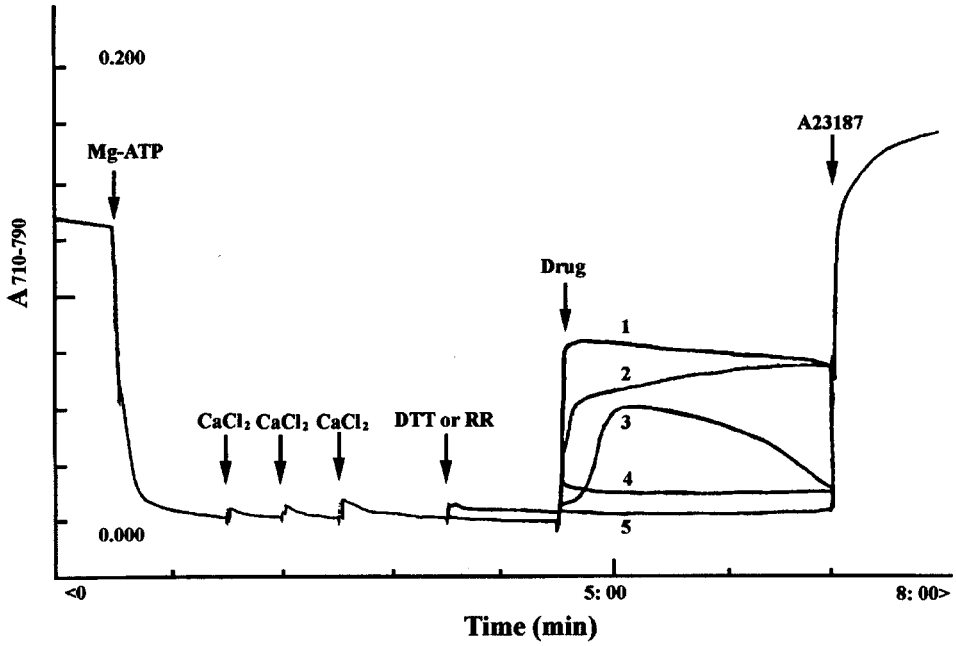

Figure 4 Induction of Ca^{2+} release by sanguinarine from SR membrane vesicles. SR vesicles (0.6 mg ml^{-1}) were actively loaded with Ca^{2+} and the Ca^{2+} concentration in the medium was monitored by absorbance difference at 710 nm and 790 nm according to the procedures outlined in Methods. Trace 1, Ca^{2+} release induced by 2 μg ml^{-1} polylysine; traces 2 and 3, Ca^{2+} release induced by 20 μM and 10 μM sanguinarine, respectively; traces 4 and 5, Ca^{2+} release induced by 20 μM sanguinarine with prior addition of 275 μM DTT and 2 μM RR, respectively.

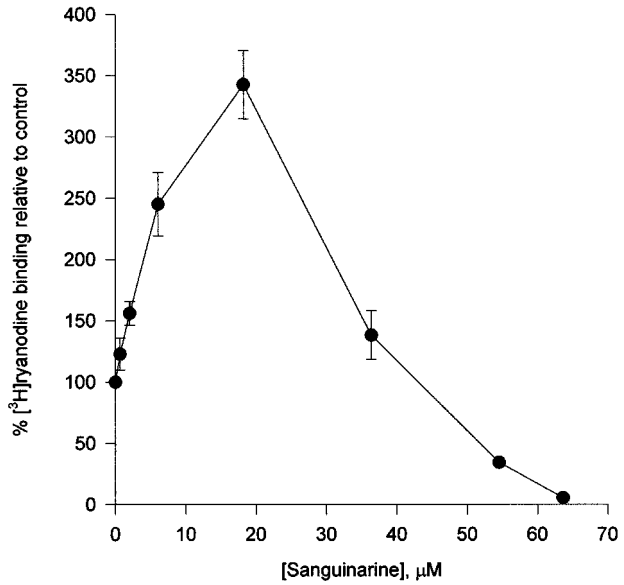

Figure 5 Dose-response effect of sanguinarine on [^{3}H]-ryanodine binding. Sanguinarine, at concentration indicated, were added to the reaction mixture containing 0.5 mg ml^{-1} SR and 10 nM [^{3}H]-ryanodine. The specific binding of [^{3}H]-ryanodine to the SR were measured according to the procedures outlined in Methods. Control binding was measured with the addition of 0.2% solvent, DMSO. Data are presented as mean ± s.e.mean of triplicate measurements from three different preparations.

diaphragm muscle. The sanguinarine effects were not affected by pretreatment with α-BuTx, an irreversible ACh receptor blocker, nor by TTX, a sodium channel blocker, suggesting that the contractures induced by sanguinarine are direct myogenic effects and independent of phrenic nerve innervation.

Pretreatment with the voltage dependent Ca^{2+} channel blocker, nifedipine, or removal of extracellular Ca^{2+} inhibited the contracture to a similar degree, suggesting that the influx of extracellular Ca^{2+} through the voltage dependent Ca^{2+} channel might be involved. Several other lines of evidence indicate the sanguinarine-induced contracture is due to specific effects of the drug on the ryanodine receptor calcium release channel of the SR. Most importantly, the sanguinarine effect was largely prevented when the internal SR Ca^{2+} pool was depleted by pretreatment with ryanodine, a specific ligand for the release channel.

We also observed that the thiol-reducing agent, DTT, counteracted the sanguinarine contractures. These data suggest that the sanguinarine effect was dependent upon key SH groups present on both the voltage dependent Ca^{2+} channel and the ryanodine receptor. It has been previously demonstrated that SH-reactive compounds induce contracture of intact skeletal muscle through the oxidation of a critical SH group of the voltage dependent Ca^{2+} channel (Oba *et al*., 1992; 1996) and the ryanodine receptor Ca^{2+} release channel of the SR (Cheng & Kang, 1998; Kang *et al*., 1998). Previous studies also showed that the effects of sanguinarine on key enzymes were caused by its ability to react with essential SH groups (Sarkar, 1948), so it appears that a unifying theme for sanguinarine action is to interfere with proteins which have essential SH groups.

The release of Ca^{2+} by SR is mediated by the ryanodine receptor which is a ligand-modulated calcium channel (Fleischer & Inui, 1989; Smith *et al*., 1985; Imagawa *et al*., 1987). A large number of chemically diverse substances have been shown to release Ca^{2+} from SR *via* the ryanodine receptor, including caffeine, Ca^{2+} itself, and ryanodine (Palade *et al*., 1989; Zucchi & Ronca-Testoni, 1997). SH reactive compounds, such as heavy metals (Salama & Ambrason, 1984; Brunder *et al*., 1988; Kang *et al*., 1997) and anthraquinones (Abramson *et al*., 1988; Cheng & Kang, 1998), were also shown to induce SR Ca^{2+} release *via* interaction with the RyR. Thus it is not unreasonable to assume that sanguinarine-induced SR Ca^{2+} release was due to the oxidation of the SH moiety of the ryanodine receptor.

The direct interaction of sanguinarine with the ryanodine receptor was further evident by its ability to modulate the [^{3}H]-ryanodine binding. The [^{3}H]-ryanodine binding was enhanced at lower concentrations of sanguinarine; however, the degree of enhancement decreased as the concentrations of sanguinarine increased. The binding was completely inhibited at higher concentrations of sanguinarine and the effects of sanguinarine at all concentrations could be reversed by DTT. These data suggest that sanguinarine can readily react with multiple classes of SH groups on the ryanodine receptor as well as other SH-reactive compounds (Abramson & Salama, 1989; Aghdasi *et al*., 1997; Kang *et al*., 1997; Feng *et al*., 1999).

It is also possible that the sanguinarine-induced contractures could be explained by the drug's inhibition of the SR Ca^{2+}-ATPase. Murphy (1976) demonstrated the presence of essential SH groups on the Ca^{2+}-ATPase. Inhibition of the ATPase could cause the SR to unload its calcium, independent of or in addition to the effects of sanguinarine on the RyR calcium release channel. It has been previously reported by Faddeeva & Beliaeva (1988) that sanguinarine can inhibit the SR Ca^{2+}-ATPase activity of skeletal muscle with IC_{50} value of 71 μM. We have also observed that the Ca^{2+}-ATPase activity in our SR membrane vesicles was inhibited by sanguinarine with a similar IC_{50} value of 80 μM. However, the contracture induced by sanguinarine reaches maximal at concentration around 50 μM and the EC_{50} is near 20 μM. This suggested that the inhibitory effect of sanguinarine on Ca^{2+}-ATPase might act in concert with the very potent effects on the RyR calcium release channel to induce contracture.

In summary, the present investigation showed that sanguinarine could induce contracture and twitch depression and hence paralysis of the isolated diaphragm. The direct effect of sanguinarine on diaphragm might be responsible for the respiratory paralysis and death of the animals from sanguinarine intoxication.

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References

ABRAMSON, J.J., BUCK, E., SALAMA, G., CASIDA, J.E. & PESSAH, I.N. (1988). Mechanism of anthraquinone-induced calcium release from skeletal muscle sarcoplasmic reticulum. *J. Biol. Chem.,* **263,** 18750 – 18758.

ABRAMSON, J.J. & SALAMA, G. (1989). Critical sulfhydryls regulate calcium release from sarcoplasmic reticulum. *J. Bioenerg Biomembr.,* **21,** 283 – 294.

AGARWAL, S., REYNOLDS, M.A., POU, S., PETERSON, D.E., CHARON, J.A. & SUZUKI, J.B. (1991). The effect of sanguinarine on human peripheral blood neutrophil viability and functions. *Oral Microbiol. Immunol.,* **6,** 51 – 61.

AGHDASI, B., ZHANG, J.Z., WU, Y., REID, M.B. & HAMILTON, S.L. (1997). Multiple classes of sulfhydryls modulate the skeletal muscle Ca^{2+} release channel. *J. Biol. Chem.,* **272,** 3739 – 3748.

BECCI, P.J., SCHWARTZ, H., BARNES, H.H. & SOUTHARD, G.L. (1987). Short-term toxicity studies of sanguinarine and of two alkaloid extracts of *Sanguinaria canadesis* L. *J. Toxicol. Environ. Health,* **20,** 199 – 208.

BRUNDER, D.G., DETTBARN, C. & PALADE, P. (1988). Heavy metal-induced Ca^{2+} release from sarcoplasmic reticulum. *J. Biol. Chem.,* **263,** 18785 – 18792.

BÜLBRING, E. (1946). Observations on the isolated phrenic nerve diaphragm preparation of the rat. *Br. J. Pharmacol. Chemother.,* **1,** 38 – 61.

CHATURVEDI, M.M., KUMAR, A., DARNAY, B.G., CHAINY, G.B.N., AGARWAL, S. & AGGARWAL, B.B. (1997). Sanguinarine (pseudochelerythrine) is a potent inhibitor of NF-κB activation, IκBα phosphorylation, and degradation. *J. Biol. Chem.,* **272,** 30129 – 30134.

CHENG, Y.W. & KANG, J.J. (1998). Emodin-induced muscle contraction of mouse diaphragm and the involvement of Ca^{2+} influx and Ca^{2+} release from sarcoplasmic reticulum. *Br. J. Pharmacol.,* **123,** 815 – 820.

CHU, A., DÍA-MUÑOZ, M., HAWKES, M.J., BRUSH, K. & HAMILTON, S.L. (1990). Ryanodine as a probe for the functional state of the skeletal muscle sarcoplasmic reticulum calcium release channel. *Mol. Pharmacol.,* **37,** 735 – 741.

CIFUENTES, M.E., RONJAT, M. & IKEMOTO, N. (1989). Polylusine induces a rapid Ca^{2+} release from sarcoplasmic reticulum vesicles by mediation of its binding to the foot protein. *Arch. Biochem. Biophys.,* **273,** 554 – 561.

DALVI, R.R. (1985). Sanguinarine: its potential as a liver toxic alkaloid present in the seeds of *Argemone mexicana*. *Experientia,* **41,** 77 – 78.

FADDEEVA, M.D. & BELIAEVA, T.N. (1988). Inhibition of the activity of membrane-bound Ca^{2+}-ATPase in the sarcoplasmic reticulum fragments of rabbit skeletal muscles by the alkaloid sanguinarine. *Tsitologiia,* **30,** 685 – 690.

FENG, W., LIU, G., XIA, R., ABRAMSON, J.J. & PESSAH, I.N. (1999). Site-selective modification of hyperreactive cysteines of ryanodine receptor complex by quinones. *Molecular Pharmaco.,* **55,** 821 – 831.

FIRATLI, E., UNAL, T., ONAN, U. & SANDALLI, P. (1994). Antioxidative activities of some chemotherapeutics. A possible mechanism in reducing gingival inflammation. *J. Clin. Periodontol.,* **21,** 680 – 683.

FLEISCHER, S. & INUI, M. (1989). Biochemistry and biophysics of excitation-contraction coupling. *Ann. Rev. Biophys. Chem.,* **18,** 333 – 364.

FLEISCHER, S., OGUNBUNMI, E.M., DIXON, M.C. & FLEER, E.A.M. (1985). Localization of Ca^{2+} release channels with ryanodine in junctional terminal cisternae of sarcoplasmic reticulum of fast skeletal muscle. *Proc. Natl. Acad. Sci. U.S.A.,* **82,** 7256 – 7259.

FRANKOS, V.H., BRUSICK, D.J., JOHNSON, E.M., MAIBACH, H.I., MUNRO, I., SQUIRE, R.A. & WEIL, C.S. (1990) Safety of sanguinaria extract as used in commercial toothpaste and oral rinse products. *J. Can. Dent. Assoc.,* **56** (supplement), 41 – 47.

GODOWSKI, K.C., WOLFF, E.D., THOMPSON, D.M., HOUSLEY, C.J., POLSON, A.M., DUNN, R.L., DUKE, S.P., STOLLER, N.H. & SOUTHARD, G.L. (1995). Whole mouth microbiota effects following subgingival delivery of sanguinarum. *J. Periodontol.,* **66,** 870 – 877.

IMAGAWA, T., SMITH, J.S., CORONADO, R. & CAMPBELL, K.P. (1987). Purified ryanodine receptor from skeletal muscle sarcoplasmic reticulum is the Ca^{2+}-permeable pore of the calcium release channel. *J. Biol. Chem.,* **262,** 16636 – 16643.

KANG, J.J., CHEN, I.L. & CHENG, Y.W. (1997). Induction of calcium release from isolated sarcoplasmic reticulum by triphenyltin. *J. Biochem (Tokyo).,* **122,** 173 – 177.

KANG, J.J., HSU, K.S. & LIN-SHIAU, S.Y. (1994). Effect of bipyridylium compounds on calcium release from triadic vesicles isolated from rabbit skeletal muscle. *Br. J. Pharmacol.,* **112,** 1216 – 1222.

KANG, J.J., LIU, S.H., CHEN, I.L., CHENG, Y.W. & LIN-SHIAU, S.Y. (1998). Comparative studies on the induction of muscle contracture in mouse diaphragm and Ca^{2+} release from sarcoplasmic reticulum vesicles by organotin compounds. *Pharmacol. Toxicol.,* **82,** 23 – 27.

LASTER, L.L. & LOBENE, R.R. (1990). New perspectives on sanguinaria clinicals: individual toothpaste and oral rinse testing. *J. Can. Dent. Assoc.,* **56** (supplement), 19 – 23.

LENFELD, J., KROUTIL, M., MARSALEK, E., SLAVIK, J., PREININGER, V. & SIMANEK, V. (1981). Antiinflammatory activity of quaternary benzophenanthridine alkaloids from *Chelidonium majus*. *Planta Med.,* **43,** 161 – 165.

MAHADY, G.B. & BEECHER, C.W.W. (1994). Quercetin-induced benzophenanthridine alkaloid production in suspension cell cultures of Sanguinaria canadensis. *Planta Med.,* **60,** 553 – 557.

MURPHY, A.J. (1976). Sulfhydryl group modification of sarcoplasmic reticulum membranes. *Biochemistry,* **15,** 4492 – 4496.

NANDI, R., MAITI, M., CHAUDHURI, K., MAHATO, S.B. & BAIRAGI, A.K. (1983). Sensitivity of vibrios to sanguinarine. *Experientia,* **39,** 524 – 525.

OBA, T., KOSHITA, M. & YAMAGUCHI, M. (1996). H_2O_2 modulates twitch tension and increases P_0 of Ca^{2+} release channel in frog skeletal muscle. *Am. J. Physiol.,* **271,** C810 – C818.

OBA, T., YAMAGUCHI, M., WANG, S. & JOHNSON, J.D. (1992). Modulation of the Ca^{2+} channel voltage sensor and excitation-contraction coupling by silver. *Biophys. J.,* **63,** 1416 – 1420.

PALADE, P. (1987). Drug-induced Ca^{2+} release from isolated sarcoplasmic reticulum. I. Use of pyrophosphate to study caffeine-induced Ca^{2+} release. *J. Biol. Chem.,* **262,** 6135 – 6141.

PALADE, P., DETTBARN, C., BRUNDER, D., STEIN, P. & HALS, G. (1989). Pharmacology of calcium release from sarcoplasmic reticulum. *Bioenerg. Biomembr.,* **21,** 295 – 320.

PARSONS, L.G., THOMAS, L.G., SOUTHARD, G.L., WOODALL, I.R. & JONES, B.J.B. (1987). Effect of sanguinaria extract on established plaque and gingivitis when supragingivally delivered as a manual rinse or under pressure in an oral irrigator. *J. Clin. Periodontol.,* **14,** 381 – 385.

PESSAH, I.N., STAMBUK, R.A. & CASIDA, J.E. (1987). Ca^{2+}-activated ryanodine binding: Mechanisms of sensitivity and intensity modulation by Mg^{2+}, caffeine and adenine nucleotides. *Mol. Pharmacol.,* **31,** 232 – 238.

SALAMA, G. & ABRAMSON, J. (1984). Silver ions trigger Ca^{2+} release by acting at the apparent physiological release site in sarcoplasmic reticulum. *J. Biol. Chem.,* **259,** 13363 – 13369.

SARKAR, S.N. (1948). Isolation from argemone oil of dihydrosanguinarine and sanguinarine: toxicity of sanguinarine. *Nature,* **162,** 265 – 266.

SCHMELLER, T., LATZ-BRUNING, B. & WINK, M. (1997). Biochemical activities of berberine, palmatine and sanguinarine mediating chemical defence against microorgnisms and herbivores. *Phytochemistry,* **44,** 257 – 266.

SEIFEN, E., ADAMS, R.J. & RIEMER, R.K. (1979). Sanguinarine: a positive inotropic alkaloid which inhibits cardiac Na^+,K^+-ATPase. *Eur. J. Pharmacol.,* **60,** 373 – 377.

SMITH, J.S., CORONADO, R. & MEISSNER, G. (1985). Sarcoplasmic reticulum contains adenine nucleotide-activated calcium channels. *Nature,* **316,** 446 – 449.

TANDON, R.K., SINGH, D.S., ARORA, R.R., LAL, P. & TANDON, B.N. (1975). Epidemic dropsy in New Delhi. *Am. J. Clin. Nutr.,* **28,** 883 – 887.

ULRICHOVÁ, J., WALTEROVÁ, D., PREININGER, V. & ŠIMÁNEK, V. (1983b). Inhibition of butyrylcholinesterase activity by some isoquinoline alkaloids. *Planta Med.,* **48,** 174 – 177.

ULRICHOVÁ, J., WALTEROVÁ, D., PREININGER, V., SLAVÍK, J., LENFELD, J., CUSHMAN, M. & ŠIMÁNEK, V. (1983a). Inhibition of acetylcholinesterase activity by some isoquinoline alkaloids. *Planta Med.,* **48,** 111 – 115.

VAVRECKOVÁ, C., GAWLIK, I. & MÜLLER, K. (1996). Benzophenanthridine alkaloids of *Chelidonium majus*; I. Inhibition of 5- and 12-lipoxygenase by a non-redox mechanism. *Planta Med.,* **62,** 397–401.

WARREN, G.B., TOON, P.A., BIRDSALL, N.J., LEE, A.G. & METCALFE, J.C. (1974). Reconstitution of a calcium pump using defined membrane components. *Proc. Natl. Acad. Sci., U.S.A.,* **71,** 622–626.

WOLFF, J. & KNIPLING, L. (1993). Antimicrotubule properties of benzophenanthridine alkaloids. *Biochemistry,* **32,** 13334–13339.

ZUCCHI, R. & RONCA-TESTONI, S. (1997). The sarcoplasmic reticulum Ca^{2+} channel/ ryanodine receptor: Modulation by endogenous effectors, drugs and disease states. *Pharmacol. Rev.,* **49,** 1–51.

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