0022-3565/00/2922-0725$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Copyright © 2000 by The American Society for Pharmacology and Experimental Therapeutics
JPET 292:725–730, 2000

Vol. 292, No. 2
Printed in U.S.A.

Bisprasin, a Novel Ca^{2+} Releaser with Caffeine-Like Properties from a Marine Sponge, *Dysidea* spp., Acts on Ca^{2+}-Induced Ca^{2+} Release Channels of Skeletal Muscle Sarcoplasmic Reticulum[1]

ATSUKO SUZUKI, KIMIHIRO MATSUNAGA, HEEJAE SHIN, JIOJI TABUDRAV, YOSHIKAZU SHIZURI, and YASUSHI OHIZUMI

Department of Pharmaceutical Molecular Biology, Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba, Aramaki, Aoba-ku, Sendai, Japan (A.S., K.M., Y.O.); and Marine Biotechnology Institute, Co., Ltd., Shimizu, Shizuoka, Japan (H.S., J.T., Y.S.)

Accepted for publication November 9, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

Bisprasin, a unique bromotyrosine derivative containing a disulfide linkage, was isolated from a marine sponge of *Dysidea* spp. This compound caused a concentration-dependent (from 10 to 30 μM) increase in the $^{45}Ca^{2+}$ release from the heavy fraction of skeletal muscle sarcoplasmic reticulum (HSR) of rabbit skeletal muscle in the same way as does caffeine. The 50% effective concentrations of bisprasin and caffeine were approximately 18 μM and 1.2 mM, respectively, indicating that the $^{45}Ca^{2+}$-releasing activity of bisprasin was approximately 70 times more potent than that of caffeine in HSR. The bell-shaped profile of Ca^{2+} dependence for bisprasin was almost the same as that for caffeine. Typical blockers of Ca^{2+}-induced Ca^{2+} release channels, such as Mg^{2+}, procaine, and ruthenium red, inhibited markedly bisprasin- and caffeine-induced $^{45}Ca^{2+}$ release from HSR. This compound, like caffeine, significantly enhanced [^{3}H]ryanodine binding to HSR. Scatchard analysis of [^{3}H]ryanodine binding to HSR revealed that bisprasin and caffeine decreased the K_D value without affecting the B_{max} value, suggesting that both the drugs facilitate the opening of ryanodine receptor channels. The bisprasin- and caffeine-induced increases in [^{3}H]ryanodine binding were further enhanced by adenosine-5′-(β,γ-methylene)triphosphate. These results suggest that the pharmacological properties of bisprasin are almost similar to those of caffeine, except for its 70-fold higher potency. Here, we present the first report on the pharmacological properties of bisprasin, which, like caffeine, induces Ca^{2+} release from skeletal muscle SR mediated through the ryanodine receptor.

The ryanodine receptor, which functions as a Ca^{2+} release channel of sarcoplasmic reticulum (SR), is postulated to play a key role in excitation-contraction coupling in the muscle (McPherson and Campbell, 1993; Coronado et al., 1994; Sutko and Airey, 1996). Genes encoding ryanodine receptors have been referred to as ryanodine receptors 1, 2, and 3. Ryanodine receptors 1 and 2 appear to be expressed predominantly in skeletal muscle and heart, respectively, whereas ryanodine receptor 3 is expressed in brain, smooth muscle, and epithelial cells (McPherson and Campbell, 1993; Sutko and Airey, 1996). Several compounds, such as amentoflavone (Suzuki et al., 1999), 2-hydroxycarbazole (Tovey et al., 1998), bastadins (Mack et al., 1994), and 9-methyl-7-bromoeudistomin D (MBED; Seino et al., 1991), have been shown to induce Ca^{2+} release from skeletal muscle SR mediated by the ryanodine receptor. Our previous reports indicated that myotoxin *a* (Ohkura et al., 1995), puff adder lectin (Ohkura et al., 1996a), and quinolidomicin A_1 (Ohkura et al., 1996b) induced Ca^{2+} release from the heavy fraction of fragmented SR (HSR) with novel properties.

Numerous marine natural products have been isolated and given much attention as useful tools for pharmacological and biological studies because of their actions on the specific sites of functional proteins (Ohizumi, 1997; Moriya et al., 1998; Strachan et al., 1999). In the course of our survey of pharmacologically active substances from natural resources, we have devoted our attention to the occurrence of natural compounds possessing Ca^{2+} releasing activity from skeletal muscle SR, because these compounds are useful as chemical probes to elucidate the functional ryanodine receptor. Recently, we successfully isolated bisprasin (Fig. 1) from a

Received for publication June 25, 1999.
[1] This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

ABBREVIATIONS: SR, sarcoplasmic reticulum; HSR, heavy fraction of fragmented skeletal muscle sarcoplasmic reticulum; LSR, light fraction of fragmented skeletal muscle sarcoplasmic reticulum; MBED, 9-methyl-7-bromoeudistomin D; MOPS, 3-(*N*-morpholino)propanesulfonic acid; AMP-PCP, adenosine-5′-(β,γ-methylene)triphosphate.

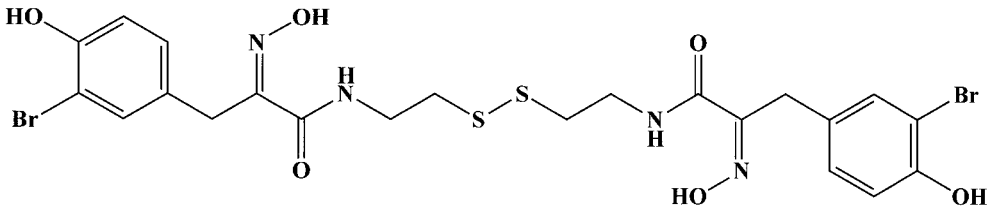

Fig. 1. Chemical structure of bisprasin.

marine sponge of *Dysidea* spp. collected at Palau. This compound is a unique brominated tyrosine-derived metabolite containing a disulfide linkage. Here, we present the first report on the pharmacological properties of bisprasin, which induces Ca^{2+} release from skeletal muscle SR mediated through the ryanodine receptor in the same way as does caffeine.

Experimental Procedures

Materials. Bisprasin was isolated from a marine sponge of *Dysidea* spp. Briefly, *Dysidea* spp. (500 g) was extracted with acetone/methanol (1:1). The extract was concentrated under reduced pressure, and the residue was partitioned between ethyl acetate and water. The ethyl acetate-soluble fraction (3.4 g) was chromatographed over silica gel with a stepwise gradient of chloroform/methanol as eluant. The fraction (1.2 g) eluted with chloroform/methanol (9:1) was subjected to the HPLC with methanol/water as eluant, resulting in the isolation of active compound (380 mg). The chemical structure of this active compound was elucidated to be bisprasin on the basis of physicochemical data such as NMR, mass, and infrared spectra (Arabshahi and Schmitz, 1987). We purchased ryanodine from S. B. Penick (New York, NY). Procaine was purchased from Sigma Chemical Co. (St. Louis, MO). $^{45}CaCl_2$ (0.7 Ci/mmol) and [^{3}H]ryanodine (60 Ci/mmol) were purchased from NEN Life Science Products. All other chemicals were of analytical grade.

Preparation of SR Vesicles from Skeletal Muscle. HSR enriched in Ca^{2+}-induced Ca^{2+} release activity was prepared from rabbit skeletal muscle as previously reported (Seino et al., 1991) with slight modification. Male rabbits (Japanese White; weight, 3 kg) were anesthetized by i.v. injection of pentobarbital sodium, and the white muscle was removed. The animals used in this study were treated in accordance with the principles and guidelines of Tohoku University Council on Animal Care. All solutions used to prepare SR membranes included protease inhibitors 76.8 mM aprotinin and 0.83 mM benzamidine. White muscle was homogenized four times with a National MX-915C mixer in 5 volumes of 5 mM Tris-maleate (pH 7.0) for 30 s at 30-s intervals. The homogenate was centrifuged at 5000*g* for 15 min. The supernatant was filtered through the cheesecloth, and the filtrate was centrifuged again at 12,000*g* for 30 min. The pellets were suspended in a solution containing 90 mM KCl and 5 mM Tris-maleate (pH 7.0) and centrifuged at 70,000*g* for 40 min. The pellets were suspended in a solution containing 90 mM KCl, 5 mM Tris-maleate (pH 7.0), and 0.3 M sucrose.

The light fraction of fragmented skeletal muscle SR (LSR) was prepared from rabbit skeletal muscle as described by Seino et al. (1991). White muscle was homogenized four times with a National MX-915C mixer in 5 volumes of 5 mM Tris-maleate (pH 7.0) for 30 s at 30-s intervals. The homogenate was centrifuged at 5000*g* for 15 min. The supernatant was filtered through the cheesecloth, and the filtrate was centrifuged at 10,000*g* for 30 min. The supernatant was centrifuged again at 70,000*g* for 50 min. The pellets were suspended in a solution containing 0.6 M KCl, 5 mM Tris-maleate (pH 7.0), and 0.3 M sucrose, and this suspension was centrifuged at 100,000*g* for 70 min. This suspension/centrifugation cycle was repeated twice. The resultant pellets were washed with a solution containing 0.1 M KCl and 5 mM Tris-maleate (pH 7.0) and resuspended in the same solution to provide LSR suspension.

The obtained SR vesicles were stored at −80°C until use. The protein concentration was determined according to the method of Bradford (1976) with BSA as a standard.

$^{45}Ca^{2+}$ Release Experiments. $^{45}Ca^{2+}$ release from the vesicular HSR passively preloaded with $^{45}Ca^{2+}$ was measured at 0°C as described previously (Nakamura et al., 1986) with slight modification. After a 12-h preincubation of 20 mg/ml HSR with 5 mM $^{45}CaCl_2$ in a solution containing 90 mM KCl and 50 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS)-Tris (pH 7.0) at 0°C, the suspension was diluted with 100 volumes of an ice-cold reaction medium containing 0.4 mM $CaCl_2$ with varying concentrations of EGTA, 90 mM KCl, and 50 mM MOPS-Tris (pH 7.0). For measurement of the amount of $^{45}Ca^{2+}$ in HSR at time 0, the suspension was diluted with the reaction medium containing 5 mM $LaCl_3$. At an appropriate time, 5 mM $LaCl_3$ was added to stop $^{45}Ca^{2+}$ release. The reaction mixture was then filtered with a Millipore filter (HAMP type, 0.45 mm pore size) and washed with 5 ml of a solution containing 5 mM $LaCl_3$, 5 mM $MgCl_2$, 90 mM KCl, and 50 mM MOPS-Tris (pH 7.0). The amount of $^{45}Ca^{2+}$ remaining in HSR vesicles was measured by counting the radioactivity on the washed filters.

The free Ca^{2+} concentration was maintained by using Ca^{2+}-EGTA buffer (0.5 mM $CaCl_2$ plus various concentrations of EGTA) and was estimated by using a microcomputer program that took into account the binding constant for Ca^{2+}-EGTA, pH, and the concentration of K^+, Mg^{2+}, and nucleotides (Sillen and Martell, 1964, 1971).

Binding Assays. [^{3}H]Ryanodine binding to HSR was examined as described previously (Furukawa et al., 1994) with slight modification. HSR (100 μg/ml) was incubated with 1 to 20 nM [^{3}H]ryanodine at 37°C for 2 h in a solution containing 0.3 M sucrose, 0.3 M KCl, 100 μM $CaCl_2$, and 20 mM Tris-HCl (pH 7.4). The amount of [^{3}H]ryanodine bound was determined by membrane filtration through Whatman filters (GF/B). Nonspecific binding was determined in the presence of 10 μM unlabeled ryanodine.

Mechanical Response. The procedure for preparing the diaphragm and the technique of measurement of contractile response were performed as described previously (Ohizumi et al., 1986). Hemidiaphragm preparations were isolated from male mice (ddys; weight, 25–30 g) and mounted in an organ bath containing 5 ml of Krebs-Ringer-bicarbonate solution of 120 mM NaCl, 4.8 mM KCl, 1.2 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25.2 mM $NaHCO_3$, and 5.8 mM glucose (pH 7.4) and were aerated with 95% O_2/5% CO_2 at 37°C. A resting tension of 1 g was applied to each preparation. Isometric contractions were measured by a force-displacement transducer and recorded on a polygraph. Preparations were stimulated directly with 5-ms pulses (supramaximal voltage) at a frequency of 0.1 Hz.

Statistical Analysis. The data are expressed as means ± S.E. Statistical comparisons were made by using Student's *t* test for unpaired data. The *n* number for statistical analysis presented the number of different preparations. $P < .05$ was considered significant.

Results

$^{45}Ca^{2+}$ Release from SR Vesicles by Bisprasin and Caffeine. The effects of bisprasin and caffeine on $^{45}Ca^{2+}$ release from HSR vesicles were measured under the conditions in which the Ca^{2+} pump did not work. Figure 2 shows the time course of the change in $^{45}Ca^{2+}$ content in HSR after the addition of bisprasin. The $^{45}Ca^{2+}$ release was markedly

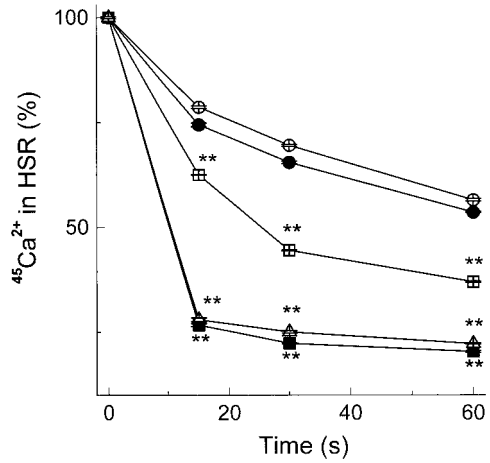

Fig. 2. The time course of stimulatory effects of bisprasin on $^{45}Ca^{2+}$ release from HSR. The $^{45}Ca^{2+}$ content in HSR vesicles was measured at 0°C by the Millipore filtration method. ○, control; ●, 10 μM bisprasin; □, 20 μM bisprasin; ■, 30 μM bisprasin; △, 100 μM bisprasin. $^{**}P < .01$, statistically significant difference from the control.

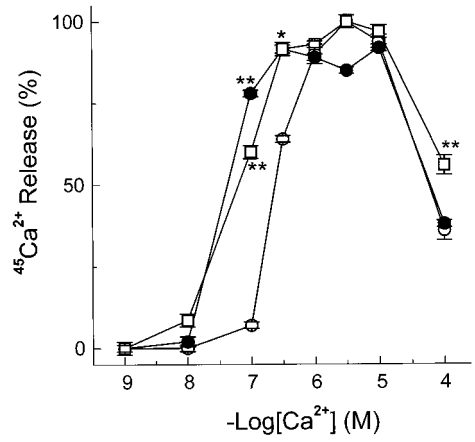

Fig. 4. Effects of free Ca^{2+} concentration on the $^{45}Ca^{2+}$ release from HSR in the absence or presence of bisprasin and caffeine. ○, control. ●, 1 mM caffeine. □, 20 μM bisprasin. $^{45}Ca^{2+}$ release was expressed as a percentage against a maximum release (100%) in the absence of bisprasin and caffeine at a Ca^{2+} concentration of 3 μM. Values are means ± S.E. ($n = 3$). $^{*}P < .05$, $^{**}P < .01$, statistically significant difference from the control.

accelerated by bisprasin over the concentration range of 10 to 100 μM. The concentration-response curve of $^{45}Ca^{2+}$ release from HSR for bisprasin and caffeine is shown in Fig. 3. $^{45}Ca^{2+}$ release was increased by bisprasin (EC_{50} = 18 μM) and caffeine (EC_{50} = 1.2 mM) in a concentration-dependent manner. In LSR, 2.1 ± 0.4 and 11.2 ± 2.5% of $^{45}Ca^{2+}$ release from HSR were induced by bisprasin (30 μM) and caffeine (1 mM), respectively.

Effects of Bisprasin and Caffeine on $^{45}Ca^{2+}$ Release from HSR over a Wide Range of Free Ca^{2+} Concentrations. $^{45}Ca^{2+}$ release was increased in a linear fashion at the free Ca^{2+} concentration from 0.1 to 1 μM (EC_{50} = 0.25 μM), reached a maximal response at 3 μM, and decreased when the free Ca^{2+} concentration was increased further from 10 to 100 μM (Fig. 4). Bisprasin (20 μM) and caffeine (1 mM) caused a parallel left shift of the concentration-response curve for $^{45}Ca^{2+}$ release plotted against the external Ca^{2+} concentration (from 1 nM to 3 μM) without affecting the maximal response (Fig. 4). At a free Ca^{2+} concentration of 100 μM, bisprasin (20 μM) stimulated $^{45}Ca^{2+}$ release, but caffeine (1 mM) did not affect it.

Effects of Typical Inhibitors of Ca^{2+}-Induced Ca^{2+} Release Channels on Bisprasin- and Caffeine-Induced $^{45}Ca^{2+}$ Release. $^{45}Ca^{2+}$ release induced by 20 μM bisprasin and 1 mM caffeine was inhibited by ruthenium red (Fig. 5), Mg^{2+} (Fig. 6), and procaine (Fig. 7) in a concentration-dependent manner. It is probable that under the present experimental conditions, there was a component refractory to the inhibition by procaine (Fig. 7).

[^{3}H]Ryanodine Binding to HSR. [^{3}H]Ryanodine binding to HSR was examined in the presence or absence of bisprasin and caffeine. As shown in Fig. 8, [^{3}H]ryanodine binding was increased by bisprasin (10 and 20 μM) and caffeine (30 mM). The degree of enhancement by bisprasin (by approximately 25%) was comparable to that of caffeine (by 25%) and MBED (by 20%, Seino et al., 1991). Figure 9 shows a saturation curve (A and C) and a corresponding Scatchard plot (B and D) of [^{3}H]ryanodine binding in the

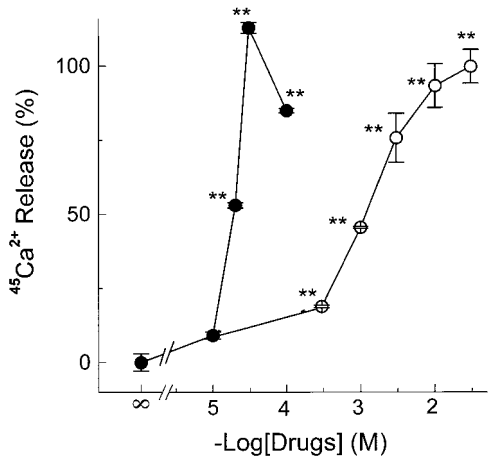

Fig. 3. Concentration-dependent acceleration of $^{45}Ca^{2+}$ release from HSR by bisprasin (●) and caffeine (○). $^{45}Ca^{2+}$ release was measured at a Ca^{2+} concentration of 0.1 μM. The amount of $^{45}Ca^{2+}$ release was calculated from the decrease in the $^{45}Ca^{2+}$ content in HSR vesicles during 1 min after dilution. Each value was obtained by subtracting the amount of $^{45}Ca^{2+}$ release measured in the absence of bisprasin from that measured in its presence. Values are means ± S.E. ($n = 3$). $^{**}P < .01$, statistically significant difference from the control.

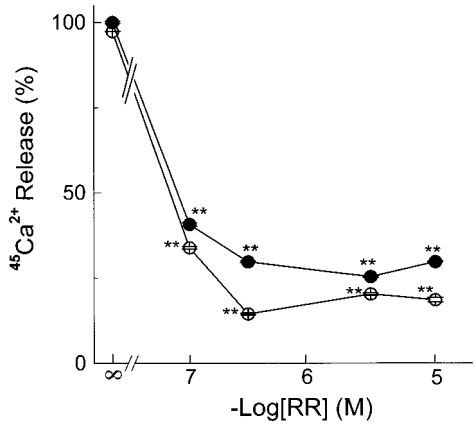

Fig. 5. Effects of ruthenium red on the $^{45}Ca^{2+}$ release induced by bisprasin and caffeine from HSR. Data are expressed as the difference between $^{45}Ca^{2+}$ release in the presence and in the absence of bisprasin and caffeine. Values are means ± S.E. ($n = 3$). ○, 1 mM caffeine. ●, 20 μM bisprasin. $^{**}P < .01$, statistically significant difference from the control (100%).

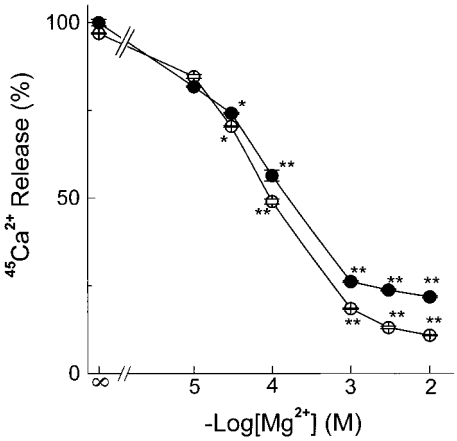

Fig. 6. Effects of Mg^{2+} on the $^{45}Ca^{2+}$ release induced by bisprasin and caffeine from HSR. Data are expressed as the difference between $^{45}Ca^{2+}$ release in the presence and in the absence of bisprasin and caffeine. Values are means ± S.E. (n = 3). ○, 1 mM caffeine. ●, 20 μM bisprasin. *P < .05, **P < .01, statistically significant difference from the control (100%).

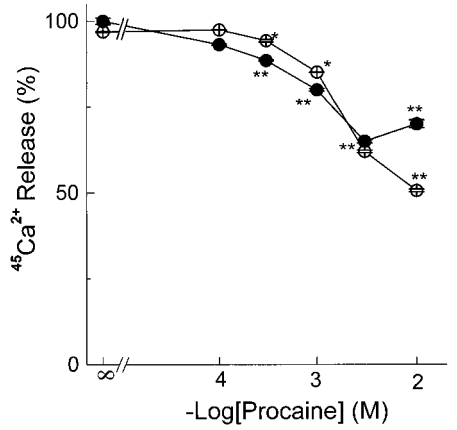

Fig. 7. Effects of procaine on the $^{45}Ca^{2+}$ release induced by bisprasin and caffeine from HSR. Data are expressed as the difference between $^{45}Ca^{2+}$ release in the presence and in the absence of bisprasin and caffeine. Values are means ± S.E. (n = 3). ○, 1 mM caffeine. ●, 20 μM bisprasin. *P < .05, **P < .01, statistically significant difference from the control (100%).

presence or absence of bisprasin (A and B) or caffeine (C and D). The K_D value was decreased from 8.33 ± 0.48 (n = 3) to 4.17 ± 0.52 (n = 3) and from 6.20 ± 0.88 (n = 3) to 4.80 ± 0.73 (n = 3) by adding bisprasin and caffeine, respectively, whereas the B_{max} value was unaffected. As shown in Fig. 10, bisprasin (20 μM)- and caffeine (30 mM)-induced increases in [^{3}H]ryanodine binding to HSR were further increased with adenosine-5′-(β,γ-methylene)triphosphate (AMP-PCP; 100 μM) by 20 and 30%, respectively.

Effects of Bisprasin on Contractile Response of Isolated Mouse Hemidiaphragm. Tetrodotoxin (1 μM), a Na^+ channel blocker, nearly abolished the contraction of hemidiaphragm induced by direct electrical stimulation, whereas bisprasin (30 μM) had no or little effect on it. KCl (50 mM)-induced contracture of hemidiaphragm was not affected by bisprasin (30 μM).

Discussion

The ryanodine receptor, which is generally known as a Ca^{2+}-induced Ca^{2+} release channel of SR (Ebashi, 1991; Sutko and Airey, 1996), may be the machinery of excitation-contraction coupling in skeletal muscle (Ford and Podolsky, 1970; Endo, 1977). Ryanodine was reported to selectively bind to its receptor in an open state. (McPherson and Campbell, 1993). The Ca^{2+} channel has been purified using [^{3}H]ryanodine as a specific ligand (Inui et al., 1987; Hymel et al., 1988; Wagenknecht et al., 1989). Not only ryanodine but also a variety of natural products, such as imperatoxin (Valdivia et al., 1992) and MBED (Seino et al., 1991), have attracted the attention of pharmacologists, physiologists, and biochemists because they act on their specific binding sites in the ryanodine receptor with high affinity. The function of Ca^{2+} release channels is inhibited by several inhibitors, such as procaine, Mg^{2+}, ruthenium red, and spermine (Palade, 1987; McPherson and Campbell, 1993).

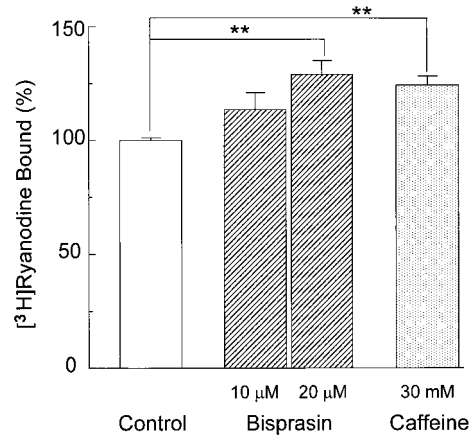

Fig. 8. Effects of bisprasin and caffeine on [^{3}H]ryanodine binding to HSR. HSR (100 μg/ml) was incubated with 2.5 nM [^{3}H]ryanodine in the presence of various concentrations of bisprasin for 2 h at 37°C. Specific binding was derived by subtracting nonspecific binding determined in the presence of 10 μM unlabeled ryanodine. Values are means ± S.E. (n = 3). **P < .01, statistically significant difference from the control.

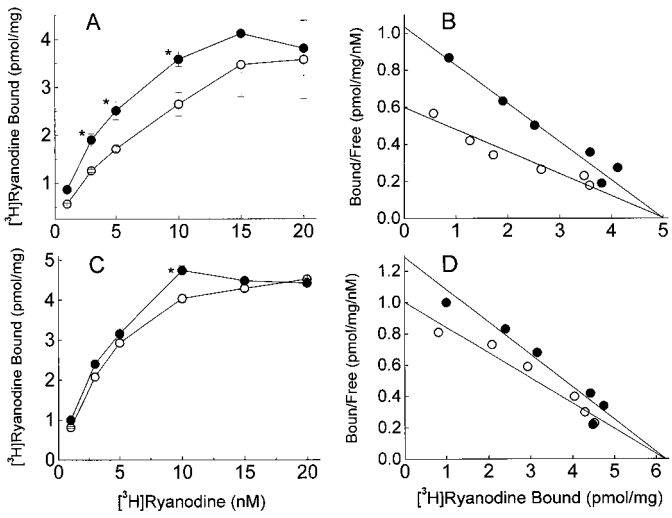

Fig. 9. A typical saturation curve (A and C) and its corresponding Scatchard plot (B and D) of [^{3}H]ryanodine binding to HSR in the presence or absence of 20 μM bisprasin (A and B) and 3 mM caffeine (C and D). HSR (100 μg/ml) was incubated with 1 to 20 nM [^{3}H]ryanodine for 2 h at 37°C in the presence or absence of 20 μM bisprasin and 3 mM caffeine. A and B: ○, control; ●, 20 μM bisprasin. C and D, ○, control; ●, 3 mM caffeine. Values are means ± S.E. (n = 3). *P < .05, statistically significant difference from the control.

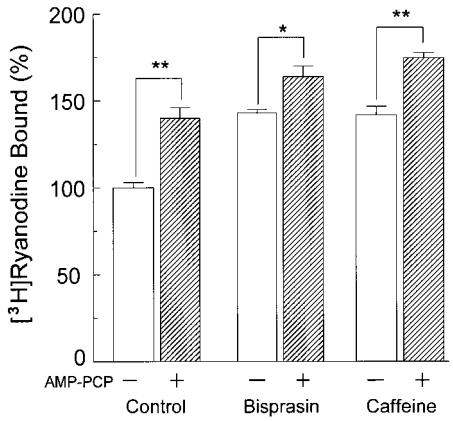

Fig. 10. Effects of AMP-PCP on the [^{3}H]ryanodine binding by bisprasin and caffeine. HSR (100 μg/ml) was incubated with 2.5 nM [^{3}H]ryanodine in the presence of various concentrations of bisprasin for 2 h at 37°C. Specific binding was derived by subtracting nonspecific binding determined in the presence of 10 μM unlabeled ryanodine. The concentrations of bisprasin, caffeine, and AMP-PCP were 20 μM, 30 mM, and 100 μM, respectively. Values are means ± S.E. (n = 3). *P < .05, **P < .01, statistically significant difference.

In our survey of natural products that exhibit Ca^{2+} releasing activity, we found that bisprasin, a unique brominated tyrosine derivative with a disulfide linkage from a marine sponge, caused a concentration-dependent increase in $^{45}Ca^{2+}$ release from HSR under the conditions in which the Ca^{2+} pump did not work. The potency of the Ca^{2+} releasing action of bisprasin from HSR was approximately 70 times greater than that of caffeine. The Ca^{2+} dependence of bisprasin-induced $^{45}Ca^{2+}$ release from HSR had a bell-shaped profile similar to that of caffeine. The $^{45}Ca^{2+}$ release induced by bisprasin and caffeine was significantly inhibited by typical blockers of Ca^{2+}-induced Ca^{2+} release channels, including procaine. These results suggest that like caffeine, bisprasin causes Ca^{2+} release by affecting Ca^{2+}-induced Ca^{2+} release channels in HSR.

[^{3}H]Ryanodine was reported to selectively bind to Ca^{2+}-induced Ca^{2+} release channels in an open state (McPherson and Campbell, 1993). In general, [^{3}H]ryanodine binding is potentiated by channel activators such as caffeine, MBED, and adenine nucleotides, whereas it is decreased by blockers of Ca^{2+}-induced Ca^{2+} release channels (Su and Chang, 1995; Ohkura et al., 1996b). [^{3}H]Ryanodine binding experiments are useful for the study of the functional state of the channel (Coronado et al., 1994). Bisprasin, like caffeine (Seino et al., 1991), markedly enhanced [^{3}H]ryanodine binding to HSR. Scatchard analysis of [^{3}H]ryanodine binding revealed that bisprasin and caffeine decreased the K_D value without affecting the B_{max} value. Bisprasin- and caffeine-induced increase in [^{3}H]ryanodine binding to HSR was further increased by AMP-PCP, an unhydrolyzable adenine nucleotide analog. These results suggest that bisprasin, like caffeine, makes it easy to open the Ca^{2+}-induced Ca^{2+} release channels, resulting in inducing Ca^{2+} release from HSR.

It has been reported that in skeletal muscle, the twitch response to direct electrical stimulation is attributed to an increasing Na^+ permeability of Na^+ channels, whereas the KCl-induced contracture is due to an increase in Ca^{2+} permeability of Ca^{2+} channels. Tetrodotoxin, a Na^+ channel blocker, nearly abolished the contraction of isolated mouse hemidiaphragm induced by direct electrical stimulation. However, even at high concentrations, bisprasin had no or little effect on it. KCl-induced contracture of hemidiaphragm was not affected by bisprasin. These results suggest that bisprasin might be a specific Ca^{2+} releaser that has no effect on Na^+ or Ca^{2+} channels. On the other hand, it is well known that LSR has far fewer ryanodine receptors than HSR (Liu et al., 1994). Bisprasin, like caffeine, slightly caused $^{45}Ca^{2+}$ release from LSR, suggesting that both of the drugs are preferential Ca^{2+} releasers in HSR.

In conclusion, the pharmacological properties of bisprasin-induced Ca^{2+} release from HSR are very similar to those of caffeine, except for its 70-fold higher potency. This is the first report on the pharmacological properties of bisprasin, which is a caffeine-like Ca^{2+} releaser in HSR that may serve as a useful biochemical tool to clarify the regulatory mechanism of Ca^{2+}-induced Ca^{2+} release in HSR.

Acknowledgments

We thank Professor P. R. Bergquist (University of Auckland) for the identification of a marine sponge.

References

Arabshahi L and Schmitz FJ (1987) Brominated tyrosine metabolites from an unidentified sponge. *J Org Chem* **52:**3584–3586.
Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72:**248–254.
Coronado R, Morrissette J, Sukhareva M and Vaughan DM (1994) Structure and function of ryanodine receptors. *Am J Physiol* **266:**C1485–C1504.
Ebashi S (1991) Excitation-contraction coupling and the mechanism of muscle contraction. *Annu Rev Physiol* **53:**1–16.
Endo M (1977) Calcium release from sarcoplasmic reticulum. *Physiol Rev* **57:**71–108.
Ford LE and Podolsky RJ (1970) Regenerative calcium release with in muscle cells. *Science (Wash DC)* **167:**58–89.
Furukawa K-I, Funayama K, Ohkura M, Oshima Y, Tu AT and Ohizumi Y (1994) Ca^{2+} release induced by myotoxin *a*, a radio-labelable probe having novel Ca^{2+} release properties in sarcoplasmic reticulum. *Br J Pharmacol* **113:**233–239.
Hymel L, Inui M, Fleischer S and Schidler H (1988) Purified ryanodine receptor of skeletal muscle sarcoplasmic reticulum from Ca^{2+}-activared oligomeric Ca^{2+} channels in planar bilayers. *Proc Natl Acad Sci USA* **86:**441–445.
Inui M, Saito A and Fleischer S (1987) Purification of the ryanodine receptor and identity with feet structure of junctional terminal cisternae of sarcoplasmic reticulum from fast skeletal muscle. *J Biol Chem* **262:**1740–1747.
Liu G, Abramson JJ, Zable AC and Pessah IN (1994) Direct evidence for the existence and functional role of hyperreactive sulfhydryls on the ryanodine receptor-triadin complex selectively labeled by the coumarin maleimide 7-diethylamino-3-(4′-maleimidylphenyl)-4-methylcoumarin. *Mol Pharmacol* **45:**189–200.
Mack MM, Molinski TF, Buck FD and Pessah IN (1994) Nobel modulators of skeletal muscle FKBP12/calcium channel complex from *Ianthella basta*. *J Biol Chem* **269:**23236–23249.
McPherson PS and Campbell KP (1993) The ryanodine receptor/Ca^{2+} release channel. *J Biol Chem* **268:**13765–13768.
Moriya T, Ishida Y, Nakamura H, Asari T, Murai A and Ohizumi Y (1998) Vasoconstriction induced by zooxanthellatoxin-B, a polyoxygenated long-chain product from a marine alga. *Eur J Pharmacol* **350:**59–65.
Nakamura Y, Kobayashi J, Gilmore J, Mascal M, Rinehart KL Jr, Nakamura H and Ohizumi Y (1986) Bromo-eudistomin D, a novel inducer of calcium release from fragmented sarcoplasmic reticulum that causes contractions of skinned muscle fibers. *J Biol Chem* **261:**4139–4142.
Ohizumi Y (1997) Application of physiologically active substances isolated from natural resources to pharmacological studies. *Jpn J Pharmacol* **73:**263–289.
Ohizumi Y, Minoshima S, Takahashi M, Kajiwara A, Nakamura H and Kobayashi J (1986) Geographutoxin II, a novel peptide inhibitor of Na channels of skeletal muscle and autonomic nerves. *J Pharmacol Exp Ther* **239:**243–248.
Ohkura M, Furukawa K-I, Oikawa K and Ohizumi Y (1995) The properties of specific binding site of ^{125}I-radioiodinated myotoxin *a*, a novel Ca^{2+} releasing agent, in skeletal muscle sarcoplasmic reticulum. *J Pharmacol Exp Ther* **273:**934–939.
Ohkura M, Miyashita Y, Kakubari M, Hayakawa Y, Seto H and Ohizumi Y (1996b) Characteristics of $^{45}Ca^{2+}$ release induced by quinolidomicin A_1, a 60-membered macrolide from skeletal muscle sarcoplasmic reticulum. *Biochim Biopys Acta* **1294:**177–182.
Ohkura M, Miyashita Y, Nikai T, Suzuki J, Komori Y, Sugihara H and Ohizumi Y (1996a) Properties of Ca^{2+} release induced by puff adder lectin, a novel lectin from the snake *Bitis arietans*, in sarcoplasmic reticulum. *J Pharmacol Exp Ther* **277:**1043–1048.
Palade P (1987) Drug-induced Ca^{2+} release from isolate sarcoplasmic reticulum. I. Use of pyrophosphate to study caffeine-induced Ca^{2+} release. *J Biol Chem* **262:**6149–6154.

Seino A, Kobayashi M, Kobayashi J, Fang Y-I, Ishibashi M, Nakamura H, Momose K and Ohizumi Y (1991) 9-Methyl-7-bromoeudistomin D, a powerful radio-labelable Ca^{2+} releaser having caffeine-like properties, acts on Ca^{2+}-induced Ca^{2+} release channels of sarcoplasmic reticulum. *J Pharmacol Exp Ther* **256:**861–867.

Sillen LG and Martell AE (1964) *Stability Constants of Metal-Ion Complexes*, The Chemical Society, London.

Sillen LG and Martell AE (1971) *Stability Constants of Metal-ion Complexes. Suppl. 1*, The Chemical Society, London.

Strachan LC, Lewis RJ and Nicholson GM (1999) Differential actions of pacific ciguatoxin-1 on sodium channel subtypes in mammalian sensory neurons. *J Pharmacol Exp Ther* **288:**379–388.

Su JY and Chang YI (1995) Modulation of the ryanodine receptor sarcoplasmic reticular Ca^{2+} channel in skinned fibers of fast- and slow-twitch skeletal muscle from rabbits. *Eur J Physiol* **460:**358–364.

Sutko JL and Airey JA (1996) Ryanodine receptor Ca^{2+} release channels: Does diversity in form equal diversity in function? *Physiol Rev* **76:**1027–1071,

Suzuki A, Matsunaga K, Mimaki Y, Sashida Y and Ohizumi Y (1999) Properties of amentoflavone, a potent caffeine-like Ca^{2+} releaser in skeletal muscle sarcoplasmic reticulum. *Eur J Pharmacol* **372:**97–102.

Tovey SC, Longland CL, Mezna M and Michelangeli F (1998) 2-Hydroxycarbazole induces Ca^{2+} release from sarcoplasmic reticulum by activating the ryanodine receptor. *Eur J Pharmacol* **354:**245–251.

Valdivia HH, Kirby MS, Lederer WJ and Coronado R (1992) Scorpion toxins targeted against the sarcoplasmic reticulum Ca^{2+}-release channel of skeletal and cardiac muscle. *Proc Natl Acad Sci USA* **89:**12185–12189.

Wagenknecht T, Grassucci R, Frank J, Saito A, Inui M and Fleisher S (1989) Three-dimensional architecture of the calcium foot structure of sarcoplasmic reticulum. *Nature (Lond)* **338:**167–170.

Send reprint requests to: Yasushi Ohizumi, Ph.D., Department of Pharmaceutical Molecular Biology, Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan. E-mail: ohizumi@mail.pharm.tohoku.ac.jp