13. R. Greeley and J. D. Iversen, *Wind as a Geologic Process* (Cambridge Univ. Press, Cambridge, 1985).
14. D. Kobayashi, *Contrib. Inst. Low Temp. Studies* **A24**, 1 (1972).
15. R. P. Sharp, *Geol. Soc. Am. Bull.* **75**, 785 (1964).
16. ———, *ibid.* **91**, 724 (1980).
17. J. L. Jensen and M. Sorenson, *Sedimentology* **33**, 547 (1986).
18. M. Sorenson, in *International Workshop on the Physics of Blown Sand*, O. E. Barndoff-Nielson *et al.*, Eds. (University of Aarhus, Aarhus, Denmark, 1986), vol. 1, pp. 141–190.
19. The motion of particles in the bed is followed by integrating numerically the equations of motion of each particle as it moves under the influence of gravity and grain-grain contact forces. Both normal and frictional forces act at each such contact, and particles may rotate in response to unbalanced torques. Because all grains in the system are followed, no artificial distinction is made between surface grains and grains below the surface. A value of 0.7 was used for the coefficient of restitution and value of 0.5 was used for the surface friction. Anecdotal numerical experiments have failed to reveal that the results are significantly dependent on these quantities (R. S. Anderson and P. K. Haff, in preparation).
20. B. T. Werner and P. K. Haff, in *Advances in Aerodynamics, Fluid Mechanics, and Hydraulics*, R. E. A. Arndt *et al.*, Eds. (American Society of Civil Engineers, New York, 1986), pp. 337–345; B. T. Werner and P. K. Haff, *Sedimentology* **35**, 189 (1988).
21. S. Mitha *et al.*, *Acta Mech.* **63**, 267 (1986).
22. B. B. Willetts and M. A. Rice, in *International Workshop on the Physics of Blown Sand*, O. E. Barndoff-Nielson *et al.*, Eds. (University of Aarhus, Aarhus, Denmark, 1986), vol. 1, pp. 83–100.
23. B. T. Werner, thesis, California Institute of Technology (1987), for single-grain impact experiments with coarse sand.
24. D. A. Rumpel, *Sedimentology* **32**, 267 (1985).
25. J. E. Ungar and P. K. Haff, *ibid.* **34**, 289 (1987).
26. The number of grains ejected, N_e (V_0), in each of several ejection-velocity bins of width dV_0, because of a single impact of velocity V_{im}, may be expressed as the sum of the number due to rebound, $N_r(V_0)$, and the number due to splash, $N_s(V_0)$, where

$$N_r(V_0) = \alpha_r \{1/[\gamma_r V_{im} (2\pi)^{1/2}]\} \cdot \exp \{-[V_0 - (d_r V_{im})]^2 / [2(\gamma_r V_{im})^2]\} dV_0$$

a normal distribution with mean = $d_r V_{im}$ and standard deviation = $\gamma_r V_{im}$ and

$$N_S(V_0) = \alpha_S V_{im} [1/d_S V_{im})] \cdot \exp [-V_0/(d_S V_{im})] dV_0$$

an exponential distribution with mean and standard deviation = $d_s V_{im}$ (see Fig. 2A). The sum over all V_0 increments must be equal to the total number of grains rebounding and splashed from the event, taken to be α_r (a constant) for the rebounds, and ($\alpha_s V_{im}$) for the splashed grains. Our results suggest that $\alpha_r = 0.95$, $d_r = 0.59$, $\gamma_r = 0.12$, $\alpha_s = 0.6$ sm^{-1}, and $d_s = 0.1$.
27. J. D. Iversen, *et al.*, *Icarus* **29**, 381 (1976).
28. R. S. Anderson, thesis, University of Washington (1986).
29. P. R. Owen, *J. Fluid. Mech.* **20**, 225 (1964).
30. A close analogy may be drawn between the saltation population ultimately limited to its steady state value by the energy available in the wind, and a biological population limited to its carrying capacity by a fixed resource base. In this analogy the probability distribution of trajectories corresponds roughly to the age structure of the biological population.
31. The response time diminishes by only 20% for a tenfold increase in α, the proportionality constant in Eq. 1, illustrating the dominant role played by the trajectory times of the high-energy trajectories (0.2 to 0.3 s) in establishing the response time.
32. We thank B. T. Werner and S. Spicklemire for sharing their insights into this problem. We also acknowledge the helpful suggestions of three anonymous reviewers. This work was supported by the U.S. Army Research Office, contract DAAL-03-86-K-0132.

12 April 1988, accepted 10 June 1988

Relaxation of Isolated Ventricular Cardiomyocytes by a Voltage-Dependent Process

JOHN H. B. BRIDGE, KENNETH W. SPITZER, PHILIP R. ERSHLER

Cell contraction and relaxation were measured in single voltage-clamped guinea pig cardiomyocytes to investigate the contribution of sarcolemmal Na^+-Ca^{2+} exchange to mechanical relaxation. Cells clamped from −80 to 0 millivolts displayed initial phasic and subsequent tonic contractions; caffeine reduced or abolished the phasic and enlarged the tonic contraction. The rate of relaxation from tonic contractions was steeply voltage-dependent and was significantly slowed in the absence of a sarcolemmal Na^+ gradient. Tonic contractions elicited in the absence of a Na^+ gradient promptly relaxed when external Na^+ was applied, reflecting activation of Na^+-Ca^{2+} exchange. It appears that a voltage-dependent Na^+-Ca^{2+} exchange can rapidly mechanically relax mammalian heart muscle.

ALTHOUGH UNIMPAIRED RELAXation is essential for normal heart function, the mechanism of relaxation in mammalian ventricular muscle is not fully understood. Relaxation continues even in the presence of caffeine. However, there is evidence that the sarcoplasmic reticulum (SR) becomes significantly more permeable to Ca^{2+} so that it cannot sequester this ion (*1–3*). Under these conditions the decline in cytosolic calcium required for relaxation may be mediated by sarcolemmal Na^+-Ca^{2+} exchange (*4*). Moreover, this relaxation should be voltage-sensitive if the exchange is voltage-sensitive. In fact, mechanical relaxation in amphibian heart, which is thought to be dependent on Na^+-Ca^{2+} exchange, displays both Na^+ (*5*) and voltage dependence (*6*). In this study we examined the contribution of Na^+-Ca^{2+} exchange to the relaxation of voltage-clamped guinea pig ventricular myocytes.

Myocytes were isolated by perfusion of guinea pig hearts with a nominally Ca^{2+}-free bicarbonate-buffered solution containing collagenase (1 mg/ml) and hyaluronidase (1 mg/ml). Cells were dispersed in a modified Tyrode's solution containing 50 μM Ca^{2+}, which was subsequently brought to 1.0 mM for cell storage. Normal Tyrode's solution contained (in millimoles per liter): 126.0 NaCl, 4.4 KCl, 5.0 $MgCl_2$, 18.0 $NaHCO_3$, 2.7 $CaCl_2$, 0.33 NaH_2PO_4, and 11.0 dextrose and was gassed with 5% CO_2 and 95% O_2. Sodium-free solution contained (in millimoles per liter): 138.0 LiCl, 4.4 KCl, 5.0 $MgCl_2$, 2.7 $CaCl_2$, Hepes 12.0, LiOH 6.4, and 11.0 dextrose. Its control solution had the same composition except that NaCl and NaOH replaced LiCl and LiOH, respectively. Hepes-buffered solutions were gassed with 100% O_2. The pH of all solutions was 7.4. Cells rested on the glass bottom of a tissue bath and were continuously bathed with Tyrode's solutions at 30° ± 0.2°C. Complete replacement of the bath solution required ≤2 seconds.

Single cells were voltage-clamped at a

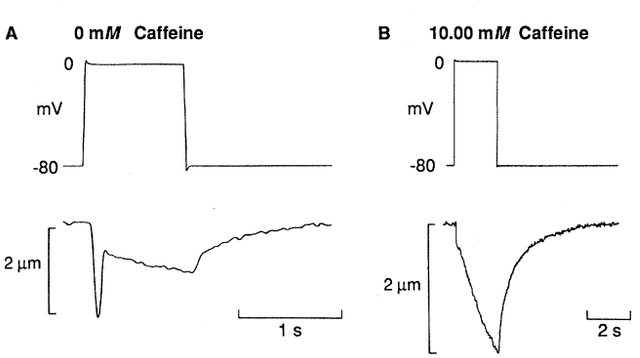

Fig. 1. Cell contraction (measured as shortening) in the absence and presence of 10.0 mM caffeine. (**A**) The cell was voltage-clamped from −80 to 0 mV for 1 second. (**B**) Another cell was exposed to 10.0 mM caffeine and subjected to a 2-second clamp of the same magnitude.

Nora Eccles Harrison Cardiovascular Research and Training Institute and the Department of Medicine, University of Utah, Salt Lake City, UT 84112.

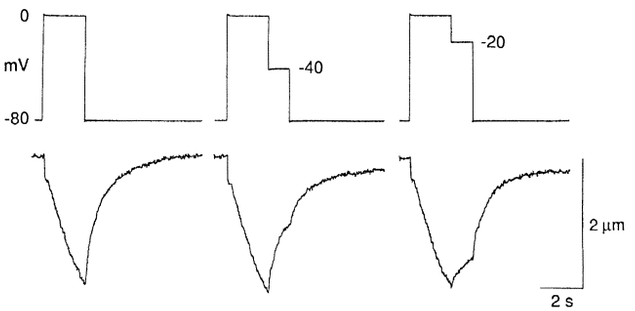

Fig. 2. Tonic contractions elicited in the presence of 10.0 m*M* caffeine by voltage-clamp pulses from −80 to 0 mV. The dependence of relaxation on voltage was measured by repolarizing to different potentials (in this example −80, −40, and −20 mV).

holding potential of −80 mV with single suction pipettes and a discontinuous voltage clamp circuit (Axoclamp-2A, Axon Instruments). Current injection was chopped at a frequency of ≥4 kHz. Suction pipettes were made of borosilicate capillary tubing, had tip diameters of 1 to 3 µm and resistances of 2 to 4 megohms, and were capacitatively shielded with Sylgard. The pipette filling solution contained (in millimoles per liter): 130 K^+ (KCl and KOH), 8.0 NaCl, 5.23 MgATP, 0.5 EGTA, 1.8 $MgCl_2$, 5.0 dextrose, and 10.0 Hepes adjusted to *p*H 7.1 with KOH. Free Ca^{2+} and Mg^{2+} concentrations were estimated to be 100 n*M* and 2.0 m*M*, respectively (*7*). In most later experiments, free Mg^{2+} was decreased to 200 µ*M*. Results obtained with this pipette solution did not alter our conclusions. However, contractions are maintained indefinitely with low Mg^{2+} in the pipette, whereas with 2.0 m*M* Mg^{2+} they tended to disappear. Cell shortening was measured with a video-based device for instantaneous length measurement (*8*).

Cells voltage-clamped from −80 to 0 mV in normal Tyrode's solution produced a phasic contraction followed by a smaller sustained tonic contraction that relaxed only on repolarization to −80 mV (Fig. 1A). Exposure to caffeine (10.0 m*M*) partially or completely eliminated the phasic component, leaving an enlarged tonic component that typically continued to increase for the duration of the pulse and relaxed on repolarization (Fig. 1B). These effects of caffeine are similar to those in voltage-clamped cardiac Purkinje strands (*9*).

We measured the voltage dependence of relaxation from tonic contractions in normal Tyrode's solution containing 10.0 m*M* caffeine (Fig. 2). The extent of relaxation varied steeply with voltage between −80 and 0 mV (Fig. 3). The processes that reduce cytosolic free Ca^{2+} and produce relaxation in caffeine-treated cells can do so only when the cell is repolarized to −80 mV. A voltage-sensitive Na^+-Ca^{2+} exchange could explain these observations. However, a voltage-sensitive Ca^{2+} leak (that is, steady-state Ca^{2+} current) might oppose and therefore slow a voltage-independent process that removes Ca^{2+} from the cytosol. Such a voltage-dependent relaxation would not necessarily reflect voltage-sensitive Na^+-Ca^{2+} exchange. To investigate these possibilities we studied the ionic dependence of relaxation.

Caffeine-treated cells were voltage-clamped in the absence of a sarcolemmal Na^+ gradient (0 Na^+ in the pipette and 0 Na^+ plus 144.4 m*M* Li^+ in the Hepes-buffered Tyrode's solution). Cells clamped from −80 to 0 mV for 3 seconds developed a large contraction. Repolarization resulted in a slow rate of relaxation (Fig. 4A) which was increased sixfold (6.0 ± 0.7 in five cells) by sudden application of 144.4 m*M* Na^+ (Fig. 4B). The slow relaxation observed in the absence of a Na^+ gradient (Fig. 4A) might result from calcium efflux via a sarcolemmal calcium pump or from residual SR calcium sequestration in 10.0 m*M* caffeine, or both effects might occur. However, these slow processes made relatively little contribution to relaxation, judging from the modest extent of relaxation following 1 second of repolarization from 0 mV to −80 mV in the absence of external sodium (that is, 15.1 ± 2.9% in five cells). In contrast, relaxation after 1 second of repolarization in the presence of the Na^+ gradient is extensive (82.8 ± 1.4% for 31 observations on 14 cells). It is this extensive relaxation which depends on the presence of a Na^+ gradient that is also steeply dependent on voltage. A voltage-insensitive Na^+-Ca^{2+} exchange opposed by a significant voltage-sensitive Ca^{2+} leak cannot explain external Na^+-dependent voltage-sensitive relaxation (Fig. 4). Between −30 and −80 mV, where relaxation displays voltage dependence, steady-state Ca^{2+} currents are inactivated. From the effect of external Na^+ and membrane potential on mechanical relaxation in caffeine-treated heart cells, we conclude that mechanical relaxation takes place when a voltage-sensitive Na^+-Ca^{2+} exchange extrudes cytosolic free Ca^{2+}. Recent intracellular cal-

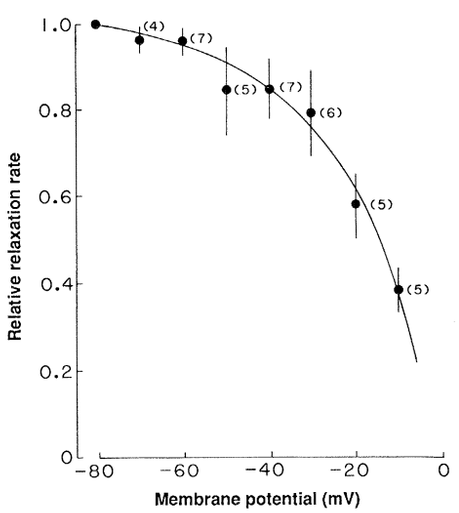

Fig. 3. Voltage dependence of relaxation rate. Because cell relengthening is not always a simple monoexponential function of time, relaxation rate has been expressed as the relative extent of relaxation 1 second after the onset of repolarization. This is in turn expressed relative to the maximum rate of relaxation, which in these experiments took place at −80 mV. The numbers in parentheses are the number of cells (and observations) contributing to each point. Values are means ± SEM. A total of 39 measurements were made on 16 cells. All results obtained in normal Tyrode's solution containing 10.0 m*M* caffeine. Line drawn by eye.

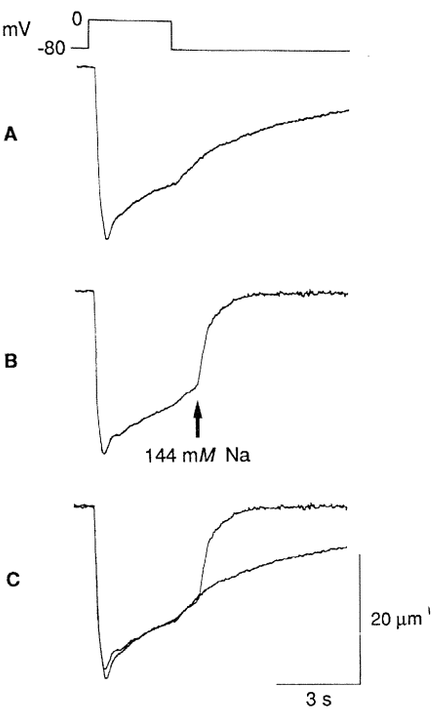

Fig. 4. Tonic contractions elicited in the absence of a Na^+ gradient with voltage-clamp pulse from −80 to 0 mV. The contractions were either maintained or (in this example) relaxed slowly during the clamp pulse. (**A**) This slow relaxation accelerated on repolarization. (**B**) Sudden application of 144.4 m*M* external Na^+ after the return to −80 mV caused an eightfold increase in relaxation rate. (**C**) Contractions in (A) and (B) are superimposed to emphasize the effect of external Na^+ on relaxation rate. All solutions contained 10.0 m*M* caffeine.

cium measurements support this view (*10*).

In normal cells, both the SR and Na^+-Ca^{2+} exchange will contribute to relaxation by removing Ca^{2+} from the cytosol. However, the Na^+-Ca^{2+} will be effective only when membrane potential (E_m) is negative to the Na^+-Ca^{2+} exchange reversal potential (E_{rev}). Owing to the inferred voltage dependence of Na^+-Ca^{2+} exchange, Ca^{2+} extrusion increases as E_m becomes increasingly negative to E_{rev}. Thus, during a single twitch the trajectory of the quantity $E_m - E_{rev}$ will determine both the onset and variation of Ca^{2+} efflux with time.

The foregoing facts will have important consequences for the regulation of contraction in heart muscle. If Ca^{2+} extrusion is abruptly delayed or reduced by prolonged membrane depolarization (for example, a prolonged action potential in which E_m spends less time negative to E_{rev}), the SR could sequester Ca^{2+} normally removed by the exchanger. Alternatively, accumulation of internal Na^+ as a result of glycoside applications would collapse $E_m - E_{rev}$, thereby reducing Ca^{2+} extrusion via the exchanger, with resulting increases in the SR Ca^{2+} pool. This enlarged SR Ca^{2+} store would presumably strengthen the subsequent contraction. In contrast, a brief depolarization (for example, shortened action potential) would have the opposite effect. Thus, the trajectory of $E_m - E_{rev}$ can regulate competition between the SR and Na^+-Ca^{2+} exchange for cytosolic Ca^{2+}. This suggests voltage-dependent control of sarcolemmal Ca^{2+} extrusion via Na^+-Ca^{2+} exchange can provide an effective and delicate mechanism for regulating the SR Ca^{2+} available for contraction.

REFERENCES AND NOTES

1. A. Weber and R. Herz, *J. Gen. Physiol.* **52**, 750 (1969); L. Blaney, H. Thomas, J. Muir, A. Henderson, *Circ. Res.* **43**, 520 (1978).
2. G. Meissner, E. Rousseau, J. LaDine, *Biophys. J.* **51**, 350a (1987).
3. A. Fabiato and F. Fabiato, *J. Physiol. (London)* **249**, 469 (1975).
4. H. Jundt, H. Porzig, H. Reuter, J. W. Stucki, *ibid.* **246**, 229 (1975).
5. M. Roulet, K. G. Mongo, G. Vassort, R. Ventura-Clapier, *Pfluegers Arch.* **379**, 359 (1979).
6. R. A. Chapman and G. C. Rodrigo, *J. Exp. Physiol.* **70**, 447 (1985).
7. A. Fabiato and F. Fabiato, *J. Physiol. (Paris)* **75**, 463 (1979).
8. B. W. Steadman, K. B. Moore, K. W. Spitzer, J. H. B. Bridge, *IEEE Trans. Biomed. Eng.* **35**, 264 (1988).
9. D. A. Eisner and M. Valdeolmillos, *J. Physiol. (London)* **364**, 313 (1985).
10. L. Barcenas-Ruiz, D. J. Beuckelmann, W. G. Wier, *Science* **238**, 1720 (1987).

11. Supported by NIH grants HL31140 and HL34288 and by awards from the American Heart Association (Utah affiliate), the Nora Eccles Treadwell Foundation, and the Richard A. and Nora Eccles Harrison Fund for Cardiovascular Research.

20 January 1988; accepted 14 June 1988

Cloning and Expression of the Human Interleukin-6 (BSF-2/IFNβ 2) Receptor

KATSUHIKO YAMASAKI, TETSUYA TAGA, YUUICHI HIRATA, HIDEO YAWATA, YOSHIKAZU KAWANISHI, BRIAN SEED, TADATSUGU TANIGUCHI, TOSHIO HIRANO, TADAMITSU KISHIMOTO

Interleukin-6 (IL-6/BSF-2/IFNβ 2) is a multifunctional cytokine that regulates the growth and differentiation of various tissues, and is known particularly for its role in the immune response and acute phase reactions. A complementary DNA encoding the human IL-6 receptor (IL-6–R) has now been isolated. The IL-6–R consists of 468 amino acids, including a signal peptide of ~19 amino acids and a domain of ~90 amino acids that is similar to a domain in the immunoglobulin (Ig) superfamily. The cytoplasmic domain of ~82 amino acids lacks a tyrosine/kinase domain, unlike other growth factor receptors.

B CELL STIMULATORY FACTOR–2 (BSF-2) was originally identified as a T cell–derived factor that causes the terminal maturation of activated B cells to Ig-producing cells (*1*). After the cDNAs were cloned, BSF-2 was found to be identical to the 26-kD protein, IFN-β 2, myeloma-plasmacytoma growth factor and hepatocyte stimulating factor (*2–6*). It is established that BSF-2, now called IL-6, has many biological functions, which include growth and differentiation activities on B cells (*1, 2, 7*), T cells (*8*), myeloma-plasma-

K. Yamasaki, T. Taga, Y. Hirata, H. Yawata, Y. Kawanishi, T. Hirano, and T. Kishimoto, Division of Immunology, Institute for Molecular and Cellular Biology, Osaka University, 1-3, Yamada-Oka, Suita, Osaka 565, Japan.
B. Seed, Department of Molecular Biology, Masssachusetts General Hospital, Boston, MA 02114.
T. Taniguichi, Division of Molecular Biology, Institute for Molecular and Cellular Biology, Osaka University, 1-3, Yamada-Oka, Suita, Osaka 565, Japan.

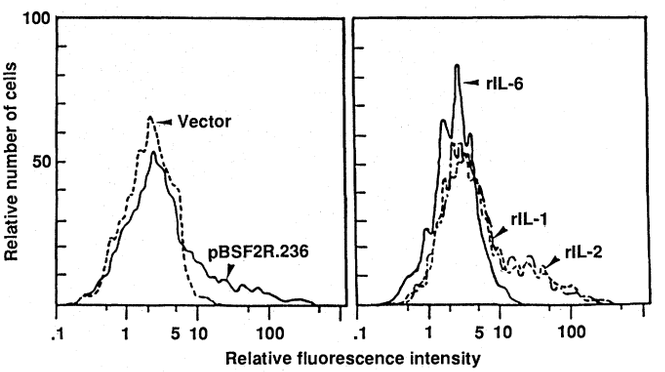

Fig. 1. Flow cytofluorometry analysis of COP cells transfected with pBSF2R.236 DNA. Left panel: murine COP cells (*24*) transfected with pBSF2R.236 DNA (——) or CDM8 vector DNA (- - -) were stained with B–rIL-6 and FITC-A as described (*13*). Right panel: COP cells transfected with pBSF2R.236 DNA were stained with ~10 ng of B–rIL-6 and FITC-A in the presence fo 200 ng of either rIL-6 (——), rIL-1 (— - — - —), or rIL-2 (- - -).

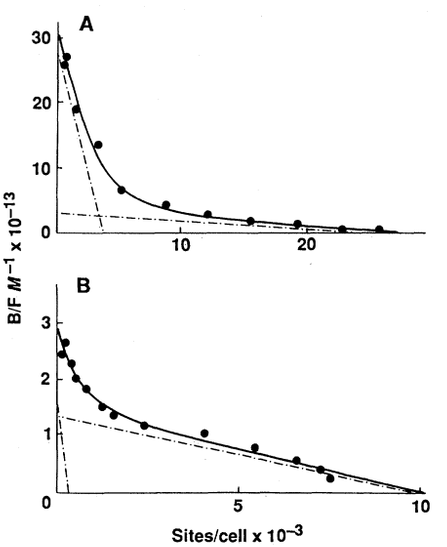

Fig. 2. Scatchard plot analysis of the IL-6–R encoded by the insert cDNA of pBSF2R.236, as well as the IL-6–R expressed on U266 cells. The IL-6–R negative human T cell line, Jurkat, was transfected with pZipNeoSVB2R [constructed by introducing the insert cDNA of pBSF2R.236 at the Bam HI site of pZipNeoSV(X)1 (*25*)] and transfectant (JBSF2R) was cloned. The IL-6 binding was assayed in both U266 (**A**) JBSF2R (**B**) as described (*14*), with ^{125}I-labeled rIL-6 (specific activity of 6.4×10^{13} cpm/g). U266, K_{d1} = 9.8 ± 2.1 p*M*, K_{d2} = 740 ± 170 p*M*, R_1 = 3000 ± 480 sites per cell, R_2 = 24,000 ± 1400 sites per cell; JBSF2R, K_{d1} = 17 ± 14 p*M*, K_{d2} = 710 ± 110 p*M*, R_1 = 240 ± 190 sites per cell, R_2 = 12,000 ± 680 sites per cell.