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CALCIUM IONOPHORE ACTIVITY OF A PROSTAGLANDIN B_1 DERIVATIVE (PGB_X)

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SUMMARY: A water soluble derivative of PGB_1, designated PGB_X, has been found to stimulate the release of Ca^{2+} from fragmented sarcoplasmic reticulum and heart mitochondria; its activity is almost two orders of magnitude greater than other prostaglandins. PGB_X demonstrates ionophoretic activity in its ability to transfer Ca^{2+} from an aqueous to an organic phase.

INTRODUCTION: It has been postulated that some of the physiological actions of prostaglandins are linked to the Ca^{2+} flux across membranes (1) and several investigators have proposed that prostaglandins have a property similar to that of ionophores (2-6). Compared with the antibiotic ionophore X537A (Hoffman-LaRoche) and A23187 (Eli Lilly Co.), however, the ionophoretic activity of prostaglandins are much weaker and it has been argued whether this property is of physiological importance. For example, the concentrations of prostaglandins necessary to demonstrate the Ca^{2+}-dependent uncoupling effect on mitochondria are much higher than those normally required for the pharmacological effects of prostaglandins (1).

Polis, Polis and Kwong (7) reported recently the synthesis of a water soluble polymeric derivative of prostaglandin B_1, designated PGB_X, which reactivates oxidative phosphorylation in aged rat liver mitochondria and prevents under prescribed conditions the uncoupling caused by Ca^{2+} and 2,4-dinitrophenol. Studies in this laboratory have confirmed the reactivation of oxidative phosphorylation by PGB_X (8). We report here that PGB_X is able to release Ca^{2+} from fragmented sarcoplasmic reticulum and heart mitochondria and that the compound has ionophoretic

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activity almost two orders of magnitude greater than other prostaglandins.

MATERIALS AND METHODS: Murexide, arsenazo III, ruthenium red, ATP and prostaglandins A_1, B_2, E_2 and $F_{1\alpha}$ were purchased from Sigma Chemical Co. (St. Louis, Missouri). MES (2-(N-morpholino) ethane sulfonic acid) and HEPES (N-2-hydroxyethyl piperazine-N'-2'ethanesulfonic acid) were purchased from Calbiochem (La Jolla, California). $[^{45}Ca^{2+}]$ was obtained from New England Nuclear (Boston, Massachusetts). A23187 was the generous gift of Dr. R. L. Hamill (Eli Lilly Col, Indianapolis, Indiana, U. S. A.). PGB_X was supplied as the sodium salt by Dr. B. D. Polis (U. S. Naval Air Development Center, Warminster, Pennsylvania, U. S. A.).

Sarcoplasmic reticulum (SR) was prepared from rabbit hind leg muscle by the method of Ogawa et al (9). Beef heart mitochondria (BHM) prepared by the method of Sordahl and Schwartz (10). Protein concentration was determined by the biuret method using bovine serum albumin as a standard.

Ca^{2+} concentration was determined by the indicator method using either murexide (11-13) or arsenazo III (14). In the case of arsenazo III, a low concentration (4 μM) was used in order to minimize the effect of the indicator-bound Ca^{2+} (12,15). Absorbance changes of these indicators were measured in an Aminco DW-2 spectrophotometer in a dual-wavelength mode. The temperature was kept at 25± 0.1°C by circulating water around the cuvette from a thermoregulated bath.

An ionophore mediated transfer of $[^{45}Ca^{2+}]$ from aqueous phase (0.25 ml; 10 mM $CaCl_2$ and 10 mM HEPES, pH 7.0) to the organic phase (1 ml; 30% butanol and 70% toluene) was measured by the method of Reed and Lardy (15).

RESULTS: As shown in Fig. 1 (A), the effect of A23187, PGB_X and three other prostaglandins on SR was measured when 90% of added Ca^{2+} was sequestered in the presence of ATP. The concentration of ATP was chosen such that the Ca^{2+} uptake would reach a steady state at that level. About a minute after reaching the steady state, the ionophore or prostaglandin was added, and the release of Ca^{2+} from SR was recorded. In the case of heart mitochondria, the respiration-driven Ca^{2+} uptake was inhibited by 5 μM ruthenium red when 95-100% of added Ca^{2+} was sequestered. A minute after, A 23187 or prostaglandin was added and the release was recorded (Fig. 1 (B)). As shown in the figures, PGB_X demonstrated strong ionophoretic activity in both cases. In SR, the order

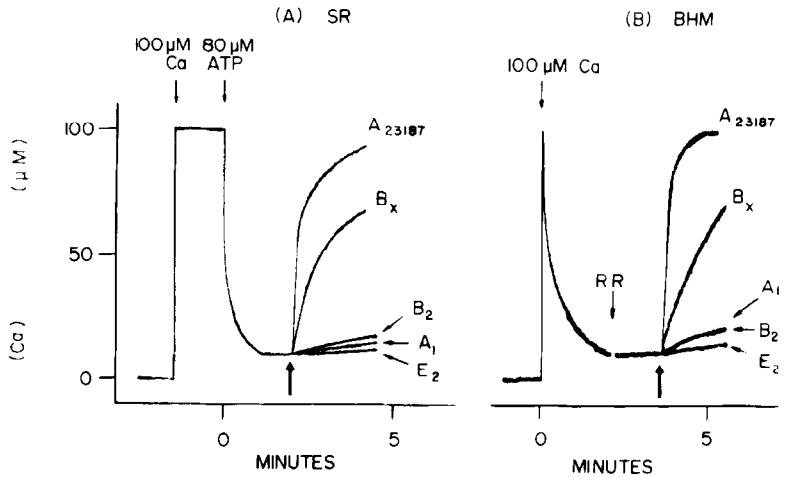

Fig. 1. (A) Effect of A23187 (0.2 μg/ml) and prostaglandins (15 μg/ml) on Ca^{2+} release from skeletal muscle SR (0.7 mg/ml). Conditions: 4 μm arsenazo III, 120 mM KCl, 5 mM $MgCl_2$, 20 mM MES (pH 7.0), at 20°C. Absorbance differences were measured at the wave-length combination of 675-685 nm. The ordinate is expressed in free Ca^{2+} concentration in SR suspension. (B) Effect of A23187 (0.2 μg/ml) and prostaglandins (15 μg/ml) on Ca^{2+} release from beef heart mitochondria (4 mg/ml). Conditions: 100 μM murexide, 300 mM sucrose, 10 mM HEPES (pH 7.0), 2 mM succinate at 25°C. 5 μM ruthenium red (RR) was added to stop Ca^{2+} uptake. The wave-length combination of 510-534 nm was used.

of ionophoretic effect of PG's was $PGB_X > B_2 > A_1 > E_2$. In beef heart mitochondria, the order was $PGB_X > A_1 \cong B_2 > E_2$.

By changing the concentration of A23187 and PG's, a dose-response relationship was measured in SR system. As shown in Fig. 2, the effect of PGB_X was 20 to 100 times greater than other PG's.

An important feature of ionophores is their ability to transfer Ca^{2+} from an aqueous phase to an organic phase. As shown in Fig. 3, PGB_X again demonstrated an ionophoretic effect almost 100 times greater than other PG's. The order of activity was $PGB_X > B_2 \cong A_1 > E_2$. $PGF_{1\alpha}$ failed to show ionophoretic activity in this solvent system.

DISCUSSION: Marmstrom and Carafoli (4) reported that prostaglandins have an ionophore-like property and release Ca^{2+} from

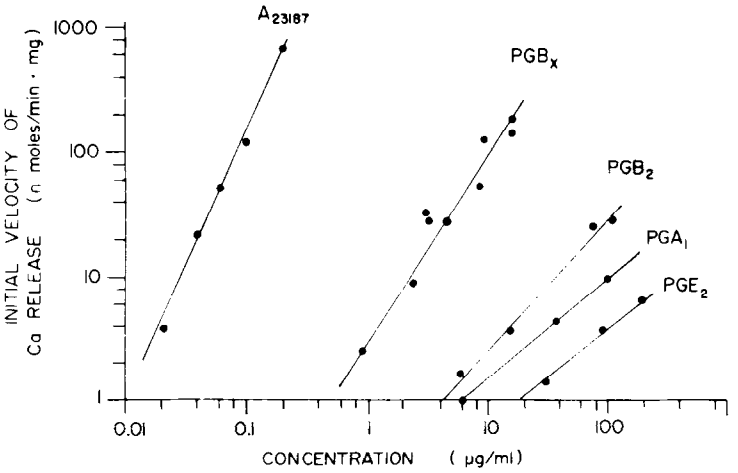

Fig. 2. Relation between concentration of A23187 and prostaglandins and the initial velocity of Ca^{2+} release from skeletal muscle SR. Conditions were the same as those in Fig. 1 (A).

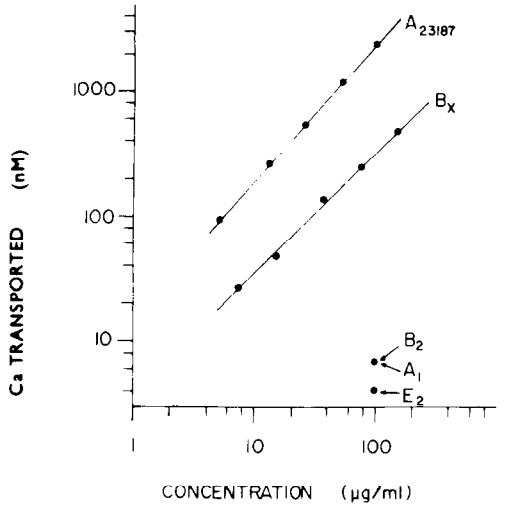

Fig. 3. Transfer of [$^{45}Ca^{2+}$] from 0.25 ml aqueous phase (10 mM $CaCl_2$, 10 mM HEPES, pH 7.0) to 1 ml organic phase (30% butanol and 70% toluene) mediated by A23187 or prostaglandins at 25°C. Concentration of A23187 and prostaglandin represents weight of chemicals/ml in organic phase.

rat liver mitochondria. We have confirmed the result using beef heart mitochondria and demonstrated the PGB_x is several orders of magnitude more effective than prostaglandins A_1, B_2 and E_2. Carsten and Miller (6) have reported recently that only PGB_2

demonstrated ionophoretic activity in a $CHCl_2$-decane/water system. According to our results, PGA_1 and PGE_2 also have some ionophoretic activity in a butanol-toluene/water system. $PGF_{1\alpha}$ did not show the property in this solvent system. The order of strength of the ionophoretic property of these prostaglandins was essentially the same in both the artificial two phase system and in beef heart mitochondria.

Unlike other prostaglandins, PGB_x is soluble in both water and organic solvents; considering the fact that ionophores are usually only soluble in organic solvents, this property of PGB_x is very unique. For the maximum transfer of cations between organic phase and aqueous phase, it is preferable for an ionophore to have affinity for both phases. Therefore, the remarkable ionophoretic activity of PGB_x may be ascribed to its affinity for both organic and aqueous phases.

For the study of ionophoretic activity of prostaglandin, mitochondria may not be the best organelle because of the complexity of their function and general instability in vitro. Sarcoplasmic reticulum seems to be more appropriate because: (a) it is a simpler organelle consisting of Ca^{2+}-transport enzymes and Ca^{2+}-binding proteins; (b) with the aid of the metallochromic indicator method, it is easy to measure the effect of prostaglandins on SR as demonstrated in this paper; (c) the preparation of SR from skeletal muscle is relatively easy; and (d) preparations can be stored at $0^{\circ}C$ for at least a week.

The relationship between the activity of PGB_x reported here and that observed by Polis et al (8) is not clear. It is possible that in aged mitochondrial preparations there is a requirement to translocate cations across the mitochondrial membrane to increase the coupling between respiration and phosphorylation or perhaps it is necessary to remove accummlated cations which could be causing uncoupling. Studies are underway to define the mode of action of PGB_x on mitochondrial phosphorylation.

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REFERENCES

1. Horton, E. W. (1969) Physiol. Rev. 49, 122-161.
2. Kirtland, S. J. and Baum, H. (1972) Nature New Biol. 236, 47-49.
3. Blondin, G. A. (1975) Ann. N. Y. Acad. Sci. 274, 98-111.
4. Malmström, K. and Carafoli, E. (1975) Arch. Biochem. Biophys. 171, 418-423.
5. Carsten, M. E. and Miller, J. D. (1977) J. Biol. Chem. 252, 1576-1581.
6. Carsten, M. E. and Miller, J. D. (1978) Arch. Biochem. Biophys. 185, 282-283.
7. Polis, B. D., Polis, E. and Kwong, S. (1979) Proc. Natl. Acad. Sci. U. S. A., 76, 1598-1602.
8. Devlin, T. M., Buccelli, B., Spees, R. and Eichel, H. J. In preparation.
9. Ogawa, Y., Harigaya, S., Ebashi, S. and Lee, K. (1971) in Methods in Pharmacol. (Schwartz, A. ed.) 1, 327-346 Appleton-Century-Crofts, New York.
10. Sordahl, L. A. and Schwartz, A. (1967) Molec. Pharmacol. 3, 509-515.
11. Ohnishi, T. and Ebashi, S. (1963) J. Biochem. 54, 506-511.
12. Ohnishi, T. and Ebashi, S. (1964) J. Biochem. 55, 599-603.
13. Ohnishi, S. T. (1978) Anal. Biochem. 85, 165-179.
14. Dipolo, R., Requena, J., Brinley, F. J., Mullins, L. J., Scarpa, A. and Tiffert, T. (1976) J. Gen. Physiol. 67, 433-467.
15. Ohnishi, S. T., (1978) Ann. N. Y. Acad. Sci. 307, 213-216.
16. Reed, P. W. and Lardy, H. A. (1972) J. Biol. Chem. 247, 6970-6977.