Vol. 126, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 16, 1985 Pages 59-62

EFFECTS OF INOSITOL-1,4,5-TRISPHOSPHATE INJECTIONS INTO SALAMANDER RODS

*Geraldine Waloga and ‡Robert E. Anderson

*Department of Physiology, Boston University School of Medicine
Boston, MA 02118

‡Cullen Eye Institute, Baylor College of Medicine
Houston, Texas 77030

Received November 19, 1984

SUMMARY: Solitary rods were isolated by trituration of salamander (Ambystoma tigrinum) retinas. One barrel of an intracellular, double-barreled micropipette was used to record membrane voltage; the other barrel was used to pressure-inject inositol-1,4,5-trisphosphate. The injection of inositol-1,4,5-trisphosphate induced a reversible hyperpolarization of the rod membrane. Injections of inositol-1,4,5-trisphosphate decreased the size of receptor potentials induced by dim lights. Conversely, light decreased the responses of the rod to injections of inositol-1,4,5-trisphosphate. These results suggest that inositol-1,4,5-trisphosphate might be involved in the modulation of rod membrane voltage during phototransduction.

A light-induced hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) has been demonstrated in the frog retina (1). One of the endproducts of this hydrolysis, inositol-1,4,5-trisphosphate (IP_3) has been shown to cause the mobilization of calcium in several tissues (2). Calcium ions have previously been implicated in the process of vertebrate phototransduction (3), and light-induced release of calcium ions from veretebrate photoreceptors has been demonstrated (4,5). Therefore, we have begun to investigate the electrophysiological effects of the intracellular injection of IP_3 into vertebrate photoreceptors.

Recently it has been demonstrated that Limulus ventral photoreceptors possess the pathways for the synthesis of PIP_2 and its subsequent light-induced

Abbreviations: PIP_2, phosphatidyl-inositol-4,5-bisphosphate; HEPES, N-2-hydroxethylpiperazine-N'-2 ethane-sulfonic acid; IP_3, inositol-1,4,5-trisphosphate; 1,4-IP_2, inositol-1,4-bisphosphate; 4,5-IP_2, inositol-4,5-bisphosphate; IP, inositol-1-phosphate; phytic acid (IP_6), inositol-hexaphosphate.

0006-291X/85 $1.50

hydrolysis to produce IP_3 (6). In addition, the intracellular pressure-injection of IP_3 near the rhabdomeric lobe of ventral photoreceptors elicits a transient depolarization of the membrane with a reversal voltage that is the same as the reversal voltage for the light-induced response (6,7). The injection of IP_3 into the rhabdomeric lobe also results in an increase in intracellular calcium ion concentration (9,10). These results are consistent with the possibility that the inositol polyphosphates play a role in invertebrate phototransduction. In this paper, we present data which suggest that the physiological effects of intracellular injections of IP_3 into vertebrate rods are also consistent with the possibility that IP_3 plays a role in the modulation of membrane voltage during vertebrate phototransduction.

MATERIALS AND METHODS

Materials: Larval salamanders (Ambystoma tigrinum) were purchased from Lowrance Waterdog Farms, Tulsa, Oklahoma. Inositol-1,4,5-trisphosphate, inositol-1,4-bisphosphate(1,4-IP_2), and inositol-4,5-bisphosphate (4,5-IP_2) were prepared from ox brain (10). Inositol-1-phosphate (IP) was prepared from beef brain (11). Inositol hexaphosphate (phytic acid) was purchased from Sigma Chemical Comapny.

Methods: Larval stage salamanders were dark-adapted for at least six hours. The retinas were dissected and transferred to a test tube under infrared light. The retinas were gently triturated in Ringer's solution that contained 108 mM NaCl, 2.4 mM KCl, 1.2 mM $MgCl_2$, 5 mM D-glucose, and 3 mM HEPES, pH 7.8. Approximately 0.5 ml of the solution containing pieces of retina was placed into a bathing chamber. The solution contained several solitary rods which settled to the bottom of the chamber and adhered to a glass surface coated with conconavalin A. The preparation was placed on the stage of an inverted microscope and superfused with oxygenated Ringer's solution. We viewed the preparation using infrared light and an infrared-sensitive television monitor. Outer segments of solitary rods were impaled with double-barreled micropipettes. One barrel was filled with a solution of either IP_3 (10-100 μM), IP_2 (100 μM), IP (100 μM) or phytic acid (100 μM) and was used for pressure-injection. The other barrel was filled with 1 M K^+ acetate and used to record membrane voltage.

RESULTS AND DISCUSSION

Light induces a reversible hyperpolarization in vertebrate rods called the receptor potential. We found that the pressure-injection of IP_3 into outer segments of dark-adapted, solitary, salamander rods also induced a reversible hyperpolarization. Injections of 1,4-IP_2, 4,5-IP_2, IP or phytic acid did not induce noticeable changes in membrane voltage. The IP_3-induced hyperpolarization was proportional to the strength of the pressure-injection, but sometimes out-

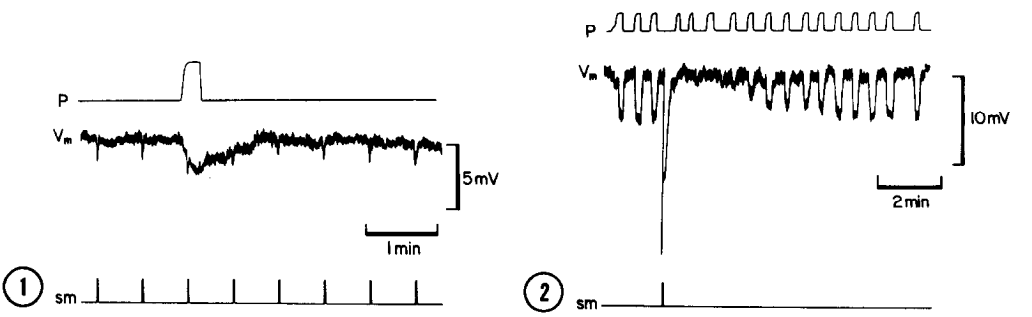

Fig. 1. Intracellular injection of IP_3 decreases the amplitude of receptor potentials. Responses of the rod induced by both light and injections of IP_3 are recorded on the membrane voltage trace (V_m). P: pressure monitor. sm: light stimulus monitor. Repetitive stimulation of the rod by uniform, dim light (6.12×10^{-13} W/cm^2), (500 nm) induced a series of hyperpolarizing receptor potentials. The pressure-injection of IP_3 into the outer segment induced a hyperpolarization that outlasted the duration of the injection. Receptor potentials elicited during the IP_3-induced hyperpolarization were diminished in amplitude. When membrane voltage recovered to its original dark level, the amplitude of the receptor potentials also recovered.

Fig. 2. Light decreases the amplitude of the responses induced by the injection of IP_3. Responses of the rod induced by both the injections of IP_3 and light are recorded on the membrane voltage trace (V_m). P: pressure monitor. sm: light stimulus monitor. Repetitive pressure-injections of IP_3 into the rod outer segment induced a series of reversible hyperpolarizations of the rod membrane. A bright light (6.12×10^{-10} W/cm^2), (500 nm) uniformly stimulated the rod for one second and induced a large receptor potential. The IP_3-induced responses were greatly attenuated after this bright light and slowly recovered as the rod dark-adapted.

lasted the duration of the applied pressure. We repetitively stimulated rods with dim light before, during and after the intracellular injection of IP_3 (Figure 1). The amplitude of the hyperpolarizing receptor potentials induced by repetitive dim lights was diminished during the time that the rod membrane was hyperpolarized by pressure-injection of IP_3. The amplitude of the receptor potentials recovered when membrane voltage returned to the level preceding the injection of IP_3.

Repetitive pressure-injection of IP_3 into the outer segments of dark-adapted rods induced repetitive, reversible hyperpolarizations (Figure 2). These IP_3-induced responses wre greatly attenuated after the rods were stimulated by a bright light. The IP_3-induced responses slowly recovered as the cell dark-adapted.

Thus, we have found that the injection of IP_3 decreases the size of receptor potentials induced by dim lights. Conversely, light decreases the

response of rods to the pressure-injection of IP_3. These electrophysiological data demonstrating the effects induced by intracellular injection of IP_3 upon rod membrane voltage, and the biochemical data demonstrating light-stimulated PIP_2 hydrolysis in frog rod outer segments, suggest that IP_3 might be involved in the modulation of membrane voltage during the process of vertebrate phototransduction.

ACKNOWLEDGEMENTS

We thank Dr. R.F. Irvine for his gifts of IP_3 and IP_2; Dr. C.E. Ballou for his gift of IP ; Drs. J.E. Brown and L.H. Pinto for helpful discussions; and Ms. Laura Tillem for help in manuscript preparation. The work was supported by NIH grants EY-04777 to G.W. and EY-00871 to R.E.A.

REFERENCES

1. Ghalayini, A. and Anderson, R.E. (1984) Biochem. Biophys. Res. Comm. 124, 503-506.
2. Berridge, M.J., and Irvine, R.F. (1984) Nature (London) (in the press).
3. Yoshikami, S. and Hagins, W.A. (1973) In Biochemistry and Physiology of Visual Pigments, ed. Langer, H., 245-256, Springer-Verlag, Berlin.
4. Gold, G.H. and Korenbrot, J.I. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 5557-5561.
5. Yoshikami, S., George, J.S., and Hagins, W.A. (1980) Nature (London) 286, 395-398.
6. Brown, J.E., Rubin, L.J., Ghalayini, A.J., Tarvar, A.P., Irvine, R.F., Berridge, M.J., and Anderson, R.E. (1984) Nature (London) 311, 160-163.
7. Fein, A., Payne, R., Corson, D.W., Berridge, M.J., and Irvine, R.F. (1984) Nature (London) 311, 157-160.
8. Brown, J.E. and Rubin, L.J. (1985) Biophys. J. (Abstract) (in the press).
9. Corson, D.W., Fein, A., and Payne, R. (1984) Biol .Bull. (Abstract) (in the press).
10. Irvine, R.F., Brown, K.D., and Berridge, M.J. (1984) Biochem. J. 222, 269-272.
11. Brockerhoff, H. and Ballou, C.E. (1961) J. Biol. Chem. 236, 1907-1911.