The mapping of the immunoglobulin genes to the precise bands involved in these translocations opens the way for further experimental tests.

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References and Notes

1. E. Max, J. Seidman, P. Leder, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3450 (1979); H. Sakano, K. Huppi, G. Heinich, S. Tonegawa, *Nature (London)* **280**, 288 (1979); J. Seidman, E. Max, P. Leder, *ibid.*, p. 370; P. Early, H. Huang, M. Davis, K. Calame, L. Hood, *Cell* **19**, 981 (1980); H. Sakano, R. Maki, Y. Kurosawa, W. Roeder, S. Tonegawa, *Nature (London)* **286**, 676 (1980).
2. For a review of information pertaining to these translocations see J. Rowley, *Science*, in press.
3. S. Ohno, M. Babonits, F. Wiener, J. Spira, G. Klein, M. Potter, *Cell* **19**, 1001 (1979).
4. G. Klein, *Nature (London)* **294**, 313 (1981).
5. C. Croce *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3416 (1979); J. Erikson, J. Martinis, C. Croce, *Nature (London)* **294**, 173 (1981); O. McBride, P. Hieter, G. Hollis, D. Swan, M. Otey, P. Leder, *J. Exp. Med.*, in press; S. Malcolm, P. Barton, D. Bentley, M. Ferguson-Smith, C. Murphy, T. Rabbitts, *Human Gene Mapping Conference VI, 1981* (National Foundation–March of Dimes, New York, in press); C. Croce *et al.*, *Ann. Hum. Genet.*, in press.
6. J. Gall and M. L. Pardue, *Proc. Natl. Acad. Sci. U.S.A.* **63**, 378 (1969); D. Gerhard, E. Kawasaki, F. Bancroft, P. Szabo, *ibid.* **78**, 3755 (1981); S. Malcolm, P. Barton, C. Murphy, M. Ferguson-Smith, *Ann. Hum. Genet.* **45**, 135 (1981).
7. M. Harper and G. Saunders, *Chromosoma* **83**, 431 (1981).
8. S. Lawrie and J. Godsen, *Hum. Genet.* **53**, 371 (1980).
9. I. R. Kirsch, unpublished data.
10. The γ4 subclone including pBR322 vector was ^{3}H-labeled by nick-translation with ^{3}H-labeled deoxyadenosine triphosphate (dATP) (46.2 Ci/mmole), ^{3}H-labeled deoxycytidine triphosphate, (dCTP) (54.1 Ci/mmole), and ^{3}H-labeled deoxythymidine triphosphate (dTTP) (94 Ci/mmole) (New England Nuclear) in the presence of excess deoxyguanosine triphosphate (dGTP). The probe was separated from unreacted ^{3}H-labeled deoxynucleotides by elution through a 12-ml Sephadex G-50 column (Pharmacia). The specific activity of the probe was 1×10^7 cpm/μg. Human peripheral blood (10 drops per flask in 10 ml of RPMI 1640 media, 20 percent fetal calf serum, glutamine, penicillin, streptomycin, and neomycin) was incubated in the presence of 0.2 ml of phytohemagglutinin (Difco) for 62 to 76 hours at 37°C (5 percent CO_2). Two hours before harvest, Colcemid (0.08 μg/ml) was added. The cells were harvested, treated with hypotonic 0.075*M* KCl, washed, fixed with methanol-acetic acid (3:1), and dropped from a height of 18 to 36 inches onto clean cold wet slides. The slides were dried in air and used for in situ hybridization within 2 weeks. The hybridization was like that described in (*7*) up to the chromosome banding. The slides were first treated with boiled ribonuclease (Sigma; 100 μg/ml) in 2 × SSC (0.3*M* NaCl, 0.03*M* disodium citrate, *p*H 7.0) and incubated at 37°C for 1 hour, rinsed four times in 2 × SSC, and dehydrated in an ethanol series. Chromosomal DNA was denatured by immersion of the slides in 70 percent formamide, 2 × SSC for 2 minutes at 70°C, and subsequently dehydrated in ethanol. The ^{3}H-labeled γ4-pBR322 probe (0.2 μg/ml) was denatured by heat (70°C) for 5 minutes [in 50 percent formamide, 2 × SSCP (0.3 ml NaCl, 0.03*M* disodium citrate, 0.04*M* $NaPO_4$, *p*H 6.0), 10 percent dextran sulfate (Pharmacia), and sonicated DNA from *Escherichia coli* (50 μg/ml)] and quickly cooled. The final *p*H was 7.0 to 7.4. Portions of the probe (25 to 30 μl) were applied to each slide. Cover slips (24 by 50 mm) were placed on the slides and the slides were incubated (12 to 18 hours at 37°C) in a sealed box with a humid environment, then rinsed three times in 50 percent formamide 2 × SSC, *p*H 7.0 at 39° to 40°C, washed five times in 2 × SSC at 39° to 40°C, and dehydrated in ethanol. Some of the slides were first stained in quinacrine dihydrochloride (Sigma Q250) (*8*); all slides were dried in air, covered with Kodak NTB2 nuclear track emulsion, and incubated in light-tight containers with desiccant at 4°C for 10 to 21 days. The slides were developed in Kodak D19 developer (1:1 with H_2O) at 15°C for 4 minutes, rinsed in twice-distilled water, treated with Kodak fixer for 5 minutes, soaked in twice-distilled water for 5 minutes, and air-dried. The dried slides were stained with quinacrine mustard (Sigma Q-2000) at a concentration of 0.005 percent in MacIlvaine's buffer (0.1*M* citric acid, 0.2*M* Na_2HPO_4, *p*H 5.4) for 20 minutes, rinsed ten times twice in MacIlvaine's buffer, and soaked an additional 10 minutes in MacIlvaine's buffer in a light-tight container. The slides were analyzed on Leitz Ortholux II microscopes equipped with incident light fluorescence capacity and 63× oil immersion fluorescent lenses with the use of the broad range blue fluorescence or with K 510 barrier filters. Grain distribution was recorded with reference to a Yunis karyogram [J. Yunis, *Hum. Pathol.* **12**, 494 (1981)] of human G- or Q-banded chromosomes at the 400-band stage.
11. J. Ravetch, U. Siebenlist, S. Korsmeyer, T. Waldmann, P. Leder, *Cell* **27**, 583 (1981).
12. A. Shimizu, N. Takahashi, Y. Yamawaki-Kataoka, T. Honjo, *Nature (London)* **289**, 149 (1981); Y. Nishida *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1581 (1981).
13. D. Cox, personal communication.
14. J. Rowley, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5729 (1977); A. Bernheim, R. Berger, G. Lenoir, *Cancer Genet. Cytogenet.* **3**, 307 (1981).
15. J. Rowley, *Annu. Rev. Genet.* **14**, 17 (1980); in *Genes, Chromosomes and Neoplasia*, F. Arrighi, P. Rao, E. Stubblefield, Eds. (Raven, New York, 1981).
16. L. Zech, U. Haglund, K. Nilsson, G. Klein, *Int. J. Cancer* **17**, 47 (1976); B. Kaiser-McCaw, A. Epstein, H. Kaplan, F. Hecht, *ibid.* **19**, 482 (1977); E. Douglass, I. Magrath, E. Lee, J. Whang-Peng, *Blood* **55**, 148 (1980).
17. W. Hayward, B. Neel, S. Astrin, *Nature (London)* **290**, 475 (1981).
18. D. Levitt and M. Cooper, *Cell* **19**, 617 (1980); P. Hieter, S. Korsmeyer, T. Waldmann, P. Leder, *Nature (London)* **290**, 368 (1981); E. Siden, P. Alt, L. Shunefeld, V. Sato, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1823 (1981); S. Korsmeyer, P. Hieter, J. Ravetch, D. Poplack, T. Waldmann, P. Leder, *ibid.*, p. 7096.
19. M. Davis *et al.*, *Nature (London)* **283**, 733 (1980); T. Kataoka, T. Kawakami, N. Takahasi, T. Honjo, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 919 (1980); R. Maki, A. Trannecker, H. Sakano, W. Roeder, S. Tonegawa, *ibid.*, p. 2138; J. Ravetch, I. Kirsch, P. Leder, *ibid.*, p. 6734.
20. I. Kirsch, J. Ravetch, S.-P. Kwan, E. Max, R. Ney, P. Leder, *Nature (London)* **293**, 585 (1981).
21. T. Maniatis *et al.*, *Cell* **15**, 687 (1978).
22. B. Hohn and K. Murray, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3259 (1977).
23. D. Rimm, D. Horness, J. Kucira, F. Blattner, *Gene* **12**, 301 (1980).
24. W. D. Benton and R. W. Davis, *Science* **196**, 180 (1977).
25. N. Obata *et al.*, *Gene* **9**, 87 (1980).
26. A. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).
27. J. Pink, S. Buttery, G. DeVries, C. Milstein, *Biochem. J.* **117**, 33 (1970).
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Role of Vitamin B_{12} in Methyl Transfer for Methane Biosynthesis by *Methanosarcina barkeri*

Abstract. *When* Methanosarcina barkeri *is grown on methanol as the sole carbon source, a B_{12}-containing protein is synthesized by this organism. This B_{12} protein contains bound aquocobalamin, and when this cofactor is reduced and methylated with [^{14}C]methyl iodide, the resultant [^{14}C]methyl B_{12} protein is extremely active in the biosynthesis of ^{14}C-labeled methane. These findings indicate that a B_{12}-dependent system is operative in the biological formation of methane in addition to other systems that are B_{12}-independent.*

Methanosarcina barkeri is one of the most metabolically diverse methane producers. In addition to producing methane from hydrogen and carbon dioxide, this organism will grow heterotrophically on methanol, methylamine, or acetate as the carbon source (*1*). The methanol metabolic pathway is

$$4CH_3OH \rightarrow 3CH_4\uparrow + CO_2\uparrow + 2H_2O$$

In 1968, Blaylock (*2*) showed that a B_{12}-containing protein was an essential component in the formation of methane from methanol. This B_{12} protein, which was partially purified from extracts of methanol-grown *Methanosarcina barkeri*, was shown to have a molecular weight of approximately 200,000; in the presence of crude extracts, it was methylated by the growth substrate methanol (*2*). The B_{12} chromophore was characteristic of bound aquocobalamin, with a maximum absorption at 352 nm.

We purified the B_{12} protein from cell extracts of methanol-grown *Methanosarcina barkeri* by elution from DEAE-52 cellulose with 0.25*M* sodium chloride, followed by chromatography on a second DEAE-52 cellulose column with 1*M* tris buffer (*p*H 7.2) as the eluting salt. After concentration by pressure dialysis, the protein was purified to homogeneity

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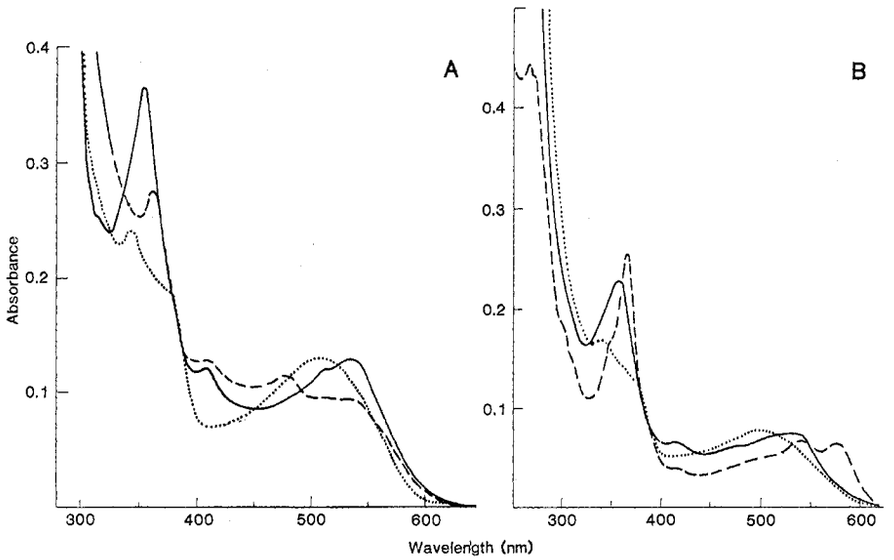
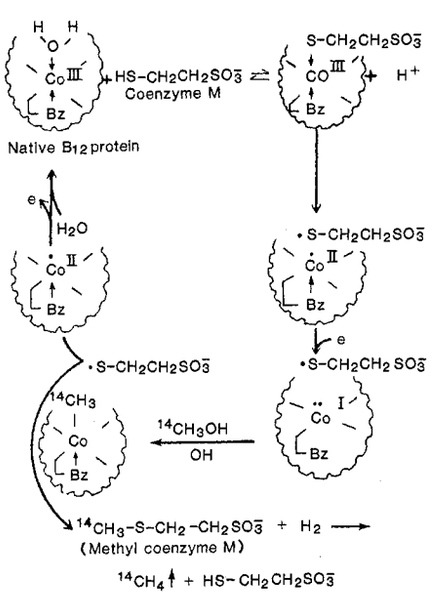


Fig. 1. (A) Ultraviolet-visible spectra of (——) native B_{12} protein, (-----) reduced B_{12} protein [cobalt(II)], and (·····) methyl B_{12} protein. (B) Ultraviolet-visible spectra of (——) native aquofactor III, (-----) cyano-factor III, and (·····) methyl-factor III.

on Sephadex G-150. With the use of several protein standards, the molecular weight of this B_{12} protein was established at approximately 200,000. Electrophoresis on sodium dodecyl sulfate–polyacrylamide gels caused the protein to dissociate into dimers (molecular weight, approximately 84,000) and monomers (molecular weight, approximately 44,000). Therefore, this B_{12} protein is similar in structure to the B_{12}-containing transmethylase that participates in the synthesis of acetic acid from methylcobalamin and pyruvate in *Clostridium thermoaceticum* (*3*). The chromophore is characteristic of bound aquocobalamin and can be reduced with mercaptoethanol or coenzyme M (ethanethiolsulfonic acid) to give a bound cobalt(II) enzyme (B_{12r} protein). The B_{12} protein can also be reduced with sodium borohydride and methylated with methyl iodide to give quantitative formation of a methyl B_{12} protein. One unusual property of the aquocobalamin-protein complex is the failure of charged ions, such as CN^-, to displace the water molecule in the sixth coordination site in 24 hours. This suggests that the water molecule is bound in an inaccessible hydrophobic environment in the protein.

When the B_{12} cofactor is removed from the protein by denaturation with ethanol, the water molecule in the sixth coordination site is rapidly displaced with CN^- to give a characteristic cyanocobalamin complex (Fig. 1B). The free coenzyme was reduced and methylated with methyl iodide, and a ^1H nuclear magnetic resonance (NMR) spectrum of this methyl corrinoid was recorded on a 300-MHz NMR spectrophotometer (Bruker). Four resonances were resolved in the aromatic region of the spectrum instead of the three protons found when 5,6-dimethylbenzimidazole is the axial base at the fifth coordination site. This suggests that the benzimidazole base is probably (5-OH)benzimidazole (factor III). When the methyl B_{12} protein was photolyzed under anaerobic conditions, a stable, low-spin cobalt(II) complex was resolved by electron paramagnetic resonance spectroscopy at 77 K. Triplets were observed from the nitrogen-hyperfine interactions of the coordinate benzimidazole base, an indication that the nucleotide base is coordinated to the cobalt when the coenzyme binds to the protein.



Scheme 1

A [^{14}C]methyl B_{12} protein was prepared by reacting [^{14}C]methyl iodide (53 μCi/mmole) with reduced B_{12} protein. The reaction rate for the formation of ^{14}C-labeled methane from this [^{14}C]-methyl B_{12} protein is at least two orders of magnitude faster than that determined for the nonenzymatic reaction between methylcobalamin and coenzyme M (*4*) (Fig. 2). Rate constants were determined for methane formation from the methyl B_{12} protein (k = 370 M^{-1} sec^{-1}), for free methylcobalamin (k = 10.6 M^{-1} sec^{-1}), and for the reaction between methylcobalamin and coenzyme M ($k = 1.1 \times 10^{-2}$ M^{-1} sec^{-1}) at *p*H 7.2 at 37°C.

In view of this large kinetic effect, it appears that the B_{12} protein functions as a methyltransferase leading to methane synthesis in methanol-grown *Methanosarcina barkeri*.

Frick *et al.* (*4*) reported the nonenzymatic transfer of a methyl group from methylcobalamin to coenzyme M. For this reaction to proceed, catalytic amounts of aquocobalamin were required to produce an active coenzyme M radical intermediate. The reaction rate was zero-order in aquocobalamin, showing the catalytic role of this molecule as an acceptor of a single electron from coenzyme M. The products of the reaction were shown to be B_{12r} [cobalt(II) B_{12}] and methyl coenzyme M. Shapiro

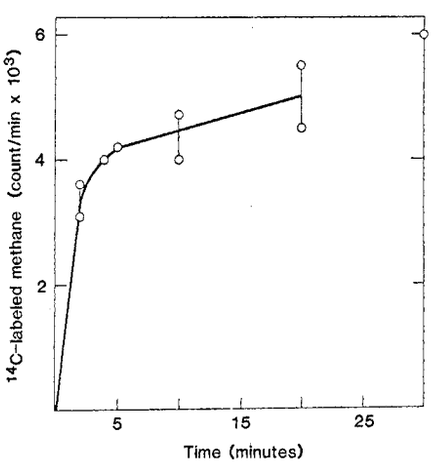


Fig. 2. Formation of ^{14}C-labeled methane from ^{14}C-labeled methyl B_{12} protein. The reaction mixture contained 5 mg of [^{14}C]methyl B_{12} protein (1617 cpm/mg), 1.5 ml of crude extracts from methanol-grown *Methanosarcina barkeri* (16.2 mg/ml), and 10 μmole of adenosine triphosphate in 0.1*M* KH_2PO_4 buffer at *p*H 7.2. The mixture was incubated at 37°C under H_2, and the reaction was stopped with the addition of 0.2 ml of 12 percent trichloroacetic acid. Duplicate reactions were performed at 2, 10, and 20 minutes. Methane was analyzed as described in (*7*).

and Wolfe (5) showed that [^{14}C]methyl coenzyme M is formed from [^{14}C]methanol, indicating a common methyl transfer route. A comparative study of the mechanisms of the nonenzymatic reaction and that of this B_{12} enzyme can now be formulated (scheme 1). This reaction mechanism explains how methyl coenzyme M is synthesized. Each of the proposed intermediates in the catalytic cycle occurs at the slower rate of the nonenzymatic reaction (4). During the last 10 years of research on the mechanism of methane biosynthesis, a role for a B_{12} cofactor has been sought, without much success. This research shows that a B_{12}-dependent methyltransferase is important in the biosynthesis of methane by *Methanosarcina barkeri*, although no similar B_{12} protein can be found in extracts of *Methanobacterium thermoautotrophicum* (6). Our results indicate that B_{12}-dependent pathways are operative in the biological formation of methane in addition to other pathways that are B_{12}-independent.

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References and Notes

1. P. J. Weimer and J. G. Zeikus, *Arch. Microbiol* **119**, 49 (1978).
2. B. A. Blaylock, *Arch. Biochem. Biophys.* **124**, 314 (1968).
3. H. G. Wood, *Vitamin B_{12}*, B. Zagalak and W. Friederich, Eds. (de Gruyter, Berlin, 1979), pp. 529–538.
4. T. Frick, M. D. Francia, J. M. Wood, *Biochim. Biophys. Acta* **428**, 808 (1976).
5. S. Shapiro and R. S. Wolfe, *J. Bacteriol.* **141**, 728 (1980).
6. D. V. Dervartanian, J. LeGall, L. E. Mortensen, H. D. Peck, unpublished data.
7. J. M. Wood, A. M. Allam, W. J. Brill, R. S. Wolfe, *J. Biol. Chem.* **240**, 4564 (1965).
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Interaction of Brain Synaptic Vesicles Induced by Endogenous Ca^{2+}-Dependent Phospholipase A_2

Abstract. *Endogenous phospholipase A_2 activity of brain synaptic vesicles was Ca^{2+}-dependent and was increased by prostaglandin $F_{2\alpha}$, calmodulin, adenosine 3′,5′-monophosphate, and adenosine triphosphate, whereas the activity was inhibited by prostaglandin E_2 in the absence or presence of calmodulin. Light-scattering measurements demonstrated that stimulation of the enzyme's activity correlated with the induction of vesicle-vesicle aggregation. The effects of these compounds on endogenous synaptic vesicle phospholipase A_2 activity may imply a common end point of their purported neuromodulatory actions, and indicate that synaptic vesicle phospholipase A_2 may play a central role in presynaptic neurotransmission.*

According to the vesicle hypothesis (1), an influx of Ca^{2+} into the presynaptic axon terminal leads to exocytosis of neurotransmitter from synaptic vesicles into the synaptic cleft. The mechanism of this Ca^{2+}-induced event is unresolved. Calcium is postulated to mediate this process directly by cross-bridging membranes (2), neutralizing membranal surface charges (3), or inducing membrane phase transitions (4). Other hypotheses of Ca^{2+}-mediated exocytosis invoke activation of brain actomyosin (5) or membrane protein phosphorylation (6).

In this study we assayed purified synaptic vesicles from bovine brain (6, 7) for phospholipase A_2 (PLA_2) (E.C. 3.1.1.4) activity using [2-^{14}C]arachidonyl phosphatidylcholine as substrate (8). When synaptic vesicles were incubated with 2.8 nmole of substrate in the presence of 1 m*M* EGTA and increasing concentrations of $CaCL_2$ (10^{-7} *M* to 10^{-1} *M*) at *p*H 9, PLA_2 activity increased with increasing Ca^{2+} concentration reaching maximum activity at 10 μ*M* $CaCl_2$. In the presence of EGTA, the amount of arachidonic acid released was 0.07 ± 0.009 nmole/mg-hour. Calcium (2 m*M*) increased the activity approximately sixfold, the amount of arachidonic acid released reaching 0.40 ± 0.13 nmole/mg-hour.

When synaptic vesicles were incubated at 37°C with increasing substrate concentrations (0.2 to 4.0 nmole) in the presence of 2 m*M* $CaCl_2$, *p*H 9.0, for 60 minutes (9), analysis of the substrate concentration curve by transformation into a Lineweaver-Burk plot revealed the Michaelis constant (K_m) to be 60 μ*M* and the maximum velocity (V_{max}), 2.0 nmole/mg-hour. Further experiments were performed under the same conditions in the presence of a variety of compounds known to be present in neurons and to modulate neurotransmission (Table 1). In no case was there a significant effect on the K_m. Calmodulin, a multifunctional Ca^{2+}-binding protein (10) reported to stimulate the release of neurotransmitter (6), caused about a fivefold increase in the enyzme's V_{max}. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which is synthesized in the brain (11) and stimulates autonomic neurotransmission (12), caused an eightfold increase in PLA_2 activity. Prostaglandin E_2 (PGE_2), which is synthesized in the brain (11), inhibits autonomic neurotransmission (12) and acts as a sedative (13), not only inhibited PLA_2 activity in the presence of Ca^{2+} alone, but also inhibited the calmodulin stimulating effect.

Individually, adenosine triphosphate (ATP) inhibited by 50 percent and adenosine 3′,5′-monophosphate (cyclic AMP), a nucleotide reported to stimulate PLA_2 activity (14), activated by 50 percent the synaptic vesicle PLA_2. However, cyclic AMP in conjunction with ATP activated PLA_2 by 200 percent. The difference in individual versus combined effects of these compounds on PLA_2 activity led us to speculate that the mechanism of the

Table 1. Effects of various conditions on the V_{max} of synaptic vesicle PLA_2. Reaction mixtures were incubated at 37°C for 60 minutes with 100 μg of synaptic vesicle protein in increasing substrate concentrations (0.2 to 4.0 nmole), brought to a final volume of 200 μl with tris buffer, *p*H 9.0, in the presence of 2 m*M* $CaCl_2$.

Conditions and concentration	V_{max} (nmole/mg-hour)	Percentage change*
Cyclic AMP (1 m*M*)	3.0	50 (+)
ATP (1 mm)	1.0	50 (−)
Cyclic AMP (1 m*M*) plus ATP (1 m*M*)	6.0	200 (+)
Calmodulin (1 μmole)	9.0	350 (+)
PGE_2 (4.0 nmole)	0.6	70 (−)
PGE_2 (4.0 nmole) plus calmodulin (1 μmole)	1.0	50 (−)
$PGF_{2\alpha}$ (4.0 nmole)	16.0	700 (+)

*Plus signs indicate stimulation and minus signs indicate inhibition.

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