Accumulation of inositol phosphates in low-passage human meningioma cells following treatment with epidermal growth factor

TOMOKI TODO, M.D., AND RUDOLF FAHLBUSCH, M.D.

Department of Neurosurgery, International Medical Center of Japan, Tokyo, Japan, and Department of Neurosurgery, University of Erlangen-Nürnberg, Erlangen, Germany

In order to elucidate some of the signal transduction processes in human meningioma cells, the authors studied the effect of epidermal growth factor (EGF) and bromocriptine on inositol phospholipid hydrolysis, using low-passage human meningioma cells in culture. Epidermal growth factor is a well-studied mitogenic factor for meningioma cells, whereas bromocriptine is known to have an inhibitory effect on meningioma cell proliferation. The addition of EGF to meningioma cells caused stimulation of inositol phosphate accumulation in a dose-dependent manner at 60 minutes posttreatment, with the maximum effect (120% to 167% of control) achieved at a concentration of 10 ng/ml. Extraction of separate inositol phosphates revealed that inositol monophosphate (IP$_1$) and inositol bisphosphate (IP$_2$), but not inositol trisphosphate (IP$_3$), accounted for the increase at 60 minutes. Kinetic analysis of EGF-stimulated inositol phospholipid hydrolysis showed that a sharp and transient increase in IP$_1$ from 5 to 12 minutes post-EGF and a transient but more gradual increase in IP$_2$ from 2 to 12 minutes post-EGF were followed by a gradual and steady increase in IP$_3$, which was significantly greater than control after 5 minutes. On the other hand, long-term studies showed a down-regulation of inositol phosphate accumulation: a 64% decrease vs. control) after 7 days of treatment with EGF (10 ng/ml). Bromocriptine (5 μM) exhibited no significant effect on inositol phosphate accumulation at 60 minutes in four of five meningiomas studied. However, of two meningiomas studied with bromocriptine in combination with EGF, both showed a significant additive increase in inositol phosphate accumulation compared to those treated with EGF alone. The results suggest a close involvement of inositol phospholipid turnover in human meningioma cells in response to mitogenic stimulation by EGF.

KEY WORDS • inositol phosphate • meningioma • epidermal growth factor • bromocriptine • cell signaling

MENINGIOMA is a common, mostly benign brain tumor that is considered to originate from arachnoid cap cells of the meninges. Several studies on the growth characteristics of human meningioma cells in culture have shown that various growth factors stimulate deoxyribonucleic acid (DNA) synthesis and cell proliferation.\textsuperscript{1,6,16,21,39,40} The most effective mitogenic factor being epidermal growth factor (EGF).\textsuperscript{1,16,39} It has been found that EGF receptors in human meningiomas are expressed regularly in low-passage meningioma cells, irrespective of their histological classification.\textsuperscript{16,25,38,40} Recently, meningioma cells have been shown to secrete a substance similar to platelet-derived growth factor (PDGF) that acts mitogenically in an autocrine manner.\textsuperscript{2} On the other hand, bromocriptine, a dopamine agonist, has been shown to inhibit the growth of meningiomas.\textsuperscript{1,29} In spite of these findings, little is known about the early intracellular biological events that occur in response to receptor stimulation in meningioma cells, although immunohistochemical studies have suggested that protein kinase C type III may play a role in meningioma cell proliferation.\textsuperscript{34}

Inositol phosphates and protein kinase C constitute two interdependent pathways in the intracellular signaling system. In response to stimulation of cell-surface receptors, hydrolysis of the membrane-localized phospholipid phosphatidylinositol 4,5-bisphosphate (PIP$_2$) is initiated, yielding two second messengers, diacylglycerol and inositol 1,4,5-trisphosphate. Diacylglycerol activates protein kinase C, whereas inositol 1,4,5-trisphosphate mediates the mobilization of calcium from internal stores.\textsuperscript{4} The hydrolysis of PIP$_2$ is catalyzed by a phosphatidylinositol-specific phospholipase C (PLC), which is commonly mediated by a guanosine triphosphate-binding protein. Phospholipase C has been shown to be composed of at least nine different isoforms.\textsuperscript{30}

The EGF receptor is one of a group of receptors that...
Inositol phosphate formation in meningioma cells

possesses intrinsic, ligand-sensitive tyrosine kinase activity.41 Although EGF has been shown in a limited number of cell types (for example, A-431 cells) to stimulate inositol phosphate formation,21,11,12,23,33,35,36 the relationship between EGF receptors and inositol phospholipid turnover was unclear until recent findings showed that stimulation of the EGF receptor results in tyrosine phosphorylation of PLC-γ.9,13,37 Alterations of the inositol phosphate signal pathway by a mitogenic or an antimiticogenic factor in human brain tumor cells has never been studied. In relation to the central nervous system, inositol phosphate accumulation has been observed in cultured rat astrocytes in response to various neurotransmitters and excitatory amino acids as well as transforming growth factor-β.19,23,24,26,27

In this paper, in order to elucidate some of the signaling processes in human meningioma cells, we have demonstrated that the accumulation of inositol phosphates is stimulated by EGF, a well-studied mitogenic factor for meningioma cells. In addition, we investigated the effect of bromocriptine on inositol phospholipid turnover as a representative inhibitory factor in meningioma cell proliferation.

Materials and Methods

Tumor Tissue

Tumor specimens were obtained from nine patients with meningioma. On based histological classification, the tumors consisted of five meningothelial meningiomas, two transitional meningiomas, one psammomatosus meningioma, and one anaplastic meningioma. The histological diagnosis was confirmed by a neuropathologist studying the original tumor tissue.

Cell Culture

Human meningioma tissue was obtained at surgery and was immediately placed in phosphate-buffered saline (PBS) containing penicillin (200 U/ml), streptomycin (200 µg/ml), and Fungizone (amphotericin B, 5 µg/ml). Under sterile conditions, the tissue was minced into 2- to 5-cm mm pieces, washed several times with the same solution to remove blood, and placed in collagenase* (200 U/ml) dissolved in 10 to 20 ml growth medium. The growth medium consisted of Eagle's minimal essential medium with Hanks' salt solution supplemented with 10% fetal calf serum (FCS), nonessential amino acids, 20 mM HEPES, sodium bicarbonate (0.075% wt/vol), glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 µg/ml). After incubation at 37°C for 24 hours, cells were mechanically dispersed, washed with fresh medium, and seeded into a 75-cm² tissue-culture flask together with 15 ml medium. Cells were grown to confluence with periodic medium changes and passages one to three times before investigation. Histological studies have demonstrated that low-passage meningioma cells retain the characteristics of precultured meningioma tissue.49

** Extraction and Separation of Inositol Phosphates

Phosphatidylinositol 4,5-bisphosphate hydrolysis was measured by a modification of the method described by Patel and Schrey.22 For short-term studies, cells were grown in 9.6-cm² six-well plates to approximately 70% confluence. Cell monolayers were washed once with PBS, then incubated for 24 hours at 37°C in growth medium containing 0.5% charcoal-stripped FCS and [³H]-inositol (5 µCi/ml). Cells were then washed twice in PBS containing 1 mM unlabeled inositol. Formation of inositol phosphates was measured between 30 seconds and 60 minutes posttreatment with EGF, and at 60 minutes after treatment with bromocriptine, in 2 ml of serum-free growth medium containing 1 mM inositol and 10 mM Li⁺ in the presence or absence of EGF and/or bromocriptine. M Mouse EGF, which has been demonstrated to bind and activate EGF receptors of human meningioma cells in culture,46 was used. Reactions were terminated by aspirating the medium and adding 1 ml of ice-cold perchloric acid (3.3% vol/vol). The wells were left on ice for a further 20 minutes, after which the supernatant was removed and transferred into chilled Eppendorf tubes together with a 400-µl water wash. The perchloric acid mixture was adjusted to pH 8.5 with 80 µl KOH (10 M) and centrifuged at 10,000 G for 2 minutes. The supernatant containing the water-soluble inositol phosphates was collected for further extraction. The cell monolayers were dissolved with 1 M NaOH after removal of the supernatant. After 10 minutes, the solution was neutralized with 1 M HCl and collected to measure phospholipids.

Inositol phosphates were extracted and separated by anion-exchange chromatography. The samples were applied to 1 ml Dowex 1-X8 (200- to 400-mesh form form) columns. Free inositol, glycerophosphoinositol, plus cyclic inositol monophosphate (IP₁) were eluted together with 7 ml water followed by 8 ml of 0.1-M ammonium formate. Inositol monophosphate, inositol bisphosphate (IP₂), and inositol trisphosphate (IP₃) were sequentially eluted with 8 ml of 0.2-M ammonium formate, 12 ml of 0.5-M ammonium formate, and 6 ml of 1.0-M ammonium formate, respectively. In some experiments, IP₁, IP₂, and IP₃ were eluted together with 10 ml of 1.0-M ammonium formate. No attempt was made to resolve isomeric forms. Samples of eluate fractions corresponding to IP₁, IP₂, IP₃, and phospholipids were counted for radioactivity using a β-scintillation counter and Ready Solv scintillation fluid. The amount of IP₁, IP₂, IP₃, and IP₃ isolated was expressed as a percentage of phospholipids.

For long-term studies, an equal number of cells were plated into 25-cm² tissue-culture flasks as described

* Collagenase obtained from Sigma-Chemie, Dienenhofen, Germany.

** Scintillation counter and Ready Solv scintillation fluid obtained from Beckman Instruments, Fullerton, California.
below. After 24 hours, cells were treated with growth medium, described above, containing 5% charcoal-stripped FCS in the presence or absence of EGF (10 ng/ml). For the EGF-treated cells, EGF was also added during prelabeling with [3H]-inositol. Extraction and separation of inositol phosphates were performed on the 7th day of treatment. Half of the flasks were used to determine cell number (see below). Inositol phospholipid hydrolysis was measured in terms of total inositol phosphates accumulated per 10^6 cells.

**Cell Growth Studies**

Cells were plated into 25-cm tissue-culture flasks in equal numbers (1 to 2 \times 10^5 cells/flask) in 3 ml of growth medium containing 10% FCS. After 24 hours' incubation at 37°C the growth medium was completely removed, and the cells were then incubated in growth medium containing 5% charcoal-stripped FCS in the absence or presence of EGF (10 ng/ml) and/or bromocriptine (5 μM). The medium was changed on the 3rd or 4th day, and cells were counted 7 days after treatment. The number of cells in each flask was determined by releasing cell nuclei in 2 ml of 10-mM HEPES buffer containing MgCl₂ (1.5 mM) with 0.2 ml Zaponin cell lysis agent and using a cell counter.

**Statistical Analysis**

All studies were performed in triplicate; that is, three cell cultures were performed for each treatment. Statistical evaluation of all data was by Student's t-test.

**Results**

**Inositol Phosphatase Response to EGF**

**Short-Term Studies.** Epidermal growth factor stimulated inositol phosphate accumulation in human meningioma cells in a dose-dependent manner at 60 minutes after treatment, with the maximum effect achieved at a concentration of 10 ng/ml. Typical results are shown in Fig. 1 left. This dose-dependent pattern of inositol phosphate accumulation corresponded to the dose-dependent stimulatory effect on meningioma cell proliferation by EGF (data not shown). In the separate extraction of IP₁, IP₂, and IP₃ at 60 minutes, significant increases in IP₁ and IP₂ by 10 ng/ml of EGF were observed, whereas no significant change was observed for IP₃ (an example is shown in Fig. 1 right). The increase in total inositol phosphate accumulation at 60 minutes following 10 ng/ml treatment with EGF ranged from 120% to 167%.

To investigate the time course of IP₁, IP₂, and IP₃ formation, a kinetic analysis of PIP₂ hydrolysis was performed by measuring each inositol phosphate component at 0.5, 2, 5, 12, and 30 minutes after treatment with or without EGF (10 ng/ml) in the presence of 10 mM LiCl. In the control study, a sharp and transient increase in IP₁ at 5 minutes and a transient but more gradual increase in IP₂ from 2 to 12 minutes were followed by a gradual and steady increase in IP₁ (Fig. 2). In EGF-stimulated cells, the duration of the transient increases in IP₁ and IP₂ value was prolonged and the peaks of the formation curves were shifted to the right, implying a longer duration for the breakdown of these molecules into IP₁. Accordingly, EGF-stimulated IP₁ accumulation showed no difference from the control values until 5 minutes posttreatment, but a significantly larger increase was observed thereafter. The time course experiment was repeated on two other meningioma cell cultures with similar results. All meningioma cells used in studies on the short-term effect of EGF were histologically classified as meningothelio-matous meningiomas.

**Long-Term Studies.** We treated human meningioma cells from two patients (meningothelio-matous meningiomas) with EGF (10 ng/ml) for 7 days to investigate the long-term response. In contrast to the short-term response, a significant suppression of total inositol phosphate accumulation was observed in the EGF-treated cells, showing a 64% decrease versus control in both meningiomas (Table 1).

**Inositol Phosphatase Response to Bromocriptine**

To investigate the effect of bromocriptine, an inhibitor of meningioma cell proliferation, on inositol phosphate formation and to compare it with the effect of
Inositol phosphate formation in meningioma cells

<table>
<thead>
<tr>
<th>Tissue Sample</th>
<th>Total Inositol Phosphate Accumulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>meningioma 1</td>
<td>1000 ± 27.7</td>
</tr>
<tr>
<td>meningioma 2</td>
<td>1000 ± 4.3</td>
</tr>
</tbody>
</table>

* Inositol phospholipid hydrolysis was measured in terms of total inositol phosphate accumulation per cell number and is expressed as a percentage of control values. Results are expressed as mean ± standard deviation of three experiments. EGF = epidermal growth factor. Significance of difference vs. control: † = p < 0.05, and ‡ = p < 0.01.

mitogenic EGF, we treated meningioma cells from five patients (one meningothelial meningioma, two transitional meningiomas, one psammomatous meningioma, and one anaplastic meningioma) with 5 μM bromocriptine for 60 minutes. In four of the five meningiomas, bromocriptine exhibited no significant effect on total inositol phosphate accumulation, but in one (the anaplastic meningioma), bromocriptine stimulated a significant increase (134% of the control). Furthermore, meningioma cells from two patients (a transitional meningioma and the anaplastic meningioma) were treated with 5 μM bromocriptine in combination with EGF (10 ng/ml). In both meningiomas, bromocriptine together with EGF produced a remarkable stimulatory effect (140% and 142% of control) and indicated a significant additive increase in inositol phosphate accumulation compared to EGF alone. The separate extractions of IP₁, IP₂, and IP₃ revealed that an increase in IP₁ mainly accounted for the increase of total inositol phosphates at 60 minutes posttreatment. The result with a transitional meningioma is shown in Fig. 3. Cell growth studies of the same two meningiomas showed that, when 5 μM bromocriptine was added in combination with 10 ng/ml of EGF, the mitogenic effect of EGF and the inhibitory effect of bromocriptine canceled each other, resulting in 18% and 11% inhibition of cell proliferation, respectively (Fig. 4).

**Discussion**

An increase in inositol phospholipid turnover has been observed in various cell types in response to numerous stimulants, most of which have receptors belonging to a guanosine triphosphate-binding protein-coupling system. With regard to mitogenic factors involving the tyrosine kinase-type receptors, the mechanism of transmembrane signaling is still unclear, although it has been shown that inositol phosphate formation is stimulated by EGF in A-431 cells and several other cell lines.⁵⁻¹³,²⁴,³⁵,³⁶ Also by PDGF in 3T3 fibroblasts,³⁶,¹⁰,¹²,¹³ On the other hand, basic fibroblast growth factor has been found to elicit cell responses in Swiss 3T3 fibroblasts without inositol phosphate accumulation.¹⁸ In the current paper, we demonstrate for
 Interestingly, EGF-stimulated phosphoinositide breakdown was quite rapid with a peak level of PtdInsP3 at 5 min in both cell types, consistent with our previous studies with PDGF and epidermal growth factor, as well as studies with other mitogens. Our results also support the notion that phosphoinositide breakdown in meningioma cells is not rate limiting upstream of PLC activation, as previously reported in breast cancer cells (MCF-7 cells). In this study, intracellular calcium mobilization was not inhibited by treatment with EGF, PDGF, or bromocriptine, suggesting that intracellular calcium mobilization is not required for the induction of phosphoinositide hydrolysis in meningioma cells.

The first time that inositol phospholipid turnover significantly increases in response to EGF in low-passage human meningioma cells. Inositol 1,4,5-trisphosphate is metabolized mainly into inositol 1,4-bisphosphate and then into inositol 4-monophosphate, although a separate pathway via inositol 1,3,4,5-tetrakisphosphate and inositol 1,3,4-trisphosphate also exists. The results of the kinetic analysis of PtdInsP3 hydrolysis showed that this sequential dephosphorylation of PtdInsP3 into PtdInsP1 occurs quite rapidly in human meningioma cells, in the order of minutes. Our results are compatible with the report by Patel and Schrey22 on bombesin-stimulated inositol phosphate accumulation in MCF-7 breast cancer cells. They found an initial transient rise in IP3 levels at 2 min posttreatment followed by a linear increase in IP3 at 5 min and thereafter. Fukami and Takenawa8 also found a rapid but transient increase in IP3 at 30 seconds posttreatment with PDGF or prostaglandin F2al in BALB/c3T3 cells. Recently, tyrosine phosphorylation of PLC by intrinsic tyrosine kinase stimulation of EGF receptors has been suggested as the mechanism for EGF-stimulated phosphoinositide hydrolysis. Interestingly, the time course of PLC-γ tyrosine phosphorylation by EGF in white blood cells also showed a rapid but transient increase, with the peak at 0.5 to 2 minutes posttreatment.14

Although our results suggest that inositol phosphates are involved in transmembrane signaling in EGF-stimulated human meningioma cells, the physiological significance is open to speculation. The inositol phosphate signal pathway is one part of a coupled signaling system following PtdInsP3 hydrolysis, the other part being controlled by diacylglycerol. Mobilization of Ca++ mediated by IP3 and protein kinase C activation by diacylglycerol generally work together to elicit a cell response. In A-431 cells, EGF-stimulated inositol phosphate accumulation has been shown to be markedly reduced in the presence of 12-O-tetradecanoylphorbol 13-acetate, suggesting a close connection between the coupled signal pathways. Presumably, EGF-stimulated IP3 hydrolysis in meningioma cells results in Ca++ mobilization and protein kinase C activation, caused by the two products IP3 and diacylglycerol, respectively, and leading to the mitogenic cell response. However, fibroblast growth factor has been shown to stimulate protein kinase C without Ca++ mobilization or inositol phosphate accumulation in 3T3 fibroblasts, implying that both of the coupled pathways are not always necessary for a cell response.18 Suzuki-Sekimori, et al.,31 showed by use of a microinjection technique in BALB 3T3 cells that an injection of diacylglycerol, but not IP3, induced DNA synthesis with the same time course as that induced by exposure of the cells to PDGF. They concluded that activation of protein kinase C is responsible for the mitogenic action of PDGF in BALB 3T3 cells. Their interesting findings give rise to the possibility that inositol phosphates may be formed only as coproducts of the protein kinase C pathway and may be without physiological significance. Several groups have shown that overexpression of

The first time that inositol phospholipid turnover significantly increases in response to EGF in low-passage human meningioma cells. Inositol 1,4,5-trisphosphate is metabolized mainly into inositol 1,4-bisphosphate and then into inositol 4-monophosphate, although a separate pathway via inositol 1,3,4,5-tetrakisphosphate and inositol 1,3,4-trisphosphate also exists. The results of the kinetic analysis of PtdInsP3 hydrolysis showed that this sequential dephosphorylation of PtdInsP3 into PtdInsP1 occurs quite rapidly in human meningioma cells, in the order of minutes. Our results are compatible with the report by Patel and Schrey22 on bombesin-stimulated inositol phosphate accumulation in MCF-7 breast cancer cells. They found an initial transient rise in IP3 levels at 2 min posttreatment followed by a linear increase in IP3 at 5 min and thereafter. Fukami and Takenawa8 also found a rapid but transient increase in IP3 at 30 seconds posttreatment with PDGF or prostaglandin F2al in BALB/c3T3 cells. Recently, tyrosine phosphorylation of PLC by intrinsic tyrosine kinase stimulation of EGF receptors has been suggested as the mechanism for EGF-stimulated phosphoinositide hydrolysis. Interestingly, the time course of PLC-γ tyrosine phosphorylation by EGF in white blood cells also showed a rapid but transient increase, with the peak at 0.5 to 2 minutes posttreatment.14

Although our results suggest that inositol phosphates are involved in transmembrane signaling in EGF-stimulated human meningioma cells, the physiological significance is open to speculation. The inositol phosphate signal pathway is one part of a coupled signaling system following PtdInsP3 hydrolysis, the other part being controlled by diacylglycerol. Mobilization of Ca++ mediated by IP3 and protein kinase C activation by diacylglycerol generally work together to elicit a cell response. In A-431 cells, EGF-stimulated inositol phosphate accumulation has been shown to be markedly reduced in the presence of 12-O-tetradecanoylphorbol 13-acetate, suggesting a close connection between the coupled signal pathways. Presumably, EGF-stimulated IP3 hydrolysis in meningioma cells results in Ca++ mobilization and protein kinase C activation, caused by the two products IP3 and diacylglycerol, respectively, and leading to the mitogenic cell response. However, fibroblast growth factor has been shown to stimulate protein kinase C without Ca++ mobilization or inositol phosphate accumulation in 3T3 fibroblasts, implying that both of the coupled pathways are not always necessary for a cell response.18 Suzuki-Sekimori, et al.,31 showed by use of a microinjection technique in BALB 3T3 cells that an injection of diacylglycerol, but not IP3, induced DNA synthesis with the same time course as that induced by exposure of the cells to PDGF. They concluded that activation of protein kinase C is responsible for the mitogenic action of PDGF in BALB 3T3 cells. Their interesting findings give rise to the possibility that inositol phosphates may be formed only as coproducts of the protein kinase C pathway and may be without physiological significance. Several groups have shown that overexpression of
Inositol phosphate formation in meningioma cells

PLC-γ in 3T3 fibroblasts resulted in increased inositol phosphate formation but not in enhanced mitogenicity in response to PDGF and basic fibroblast growth factor, suggesting that PLC-γ-mediated phosphoinositide metabolism may not be the limiting mechanism in the transmembrane signaling initiated by these growth factors. Whether the increased inositol phospholipid turnover in response to EGF in human meningioma cells plays a physiologically significant role in transmembrane signaling awaits further studies.

In contrast to the increased inositol phosphate accumulation observed promptly after stimulation by EGF, a significant decrease was observed in meningioma cells after 7 days of EGF treatment in the long-term studies. The results showed that with chronic EGF stimulation the inositol phospholipid turnover in meningioma cells becomes down-regulated. It has been suggested that protein kinase C exerts negative-feedback control over various steps of the cell signaling process. The receptor for EGF has been shown to be phosphorylated by protein kinase C, resulting in a rapid decrease in high-affinity binding of EGF as well as inhibition of the ligand-induced tyrosine phosphorylation. Whether this is also the case in meningioma cells remains speculative.

Although bromocriptine alone did not stimulate inositol phosphate formation in four of five meningiomas studied, bromocriptine in combination with EGF induced a significant additive increase in inositol phosphate accumulation compared to the increase by EGF alone in both of two meningiomas studied. In cell growth studies, treatment with bromocriptine and EGF together resulted in a small inhibition of cell proliferation. Bromocriptine has been shown to have an inhibitory effect on meningioma cell proliferation through stimulation of dopamine D2 receptors. Our observations show that bromocriptine does not inhibit cell proliferation by suppressing transmembrane inositol phosphate signaling initiated by mitogenic factors; rather, our results imply that EGF receptors and dopamine D2 receptors utilize separate signaling systems within a meningioma cell. The effect of bromocriptine does not seem to be directly mediated by the inositol phosphate pathway. An alternative interpretation for our observations of EGF and bromocriptine may be that protein kinase C is more centrally involved than are inositol phosphates in the mitogenic effect of EGF, as has been shown in other cells.

To our knowledge, no other study has examined the effect of simultaneous doses of a mitogenic factor and an inhibitor of inositol phospholipid turnover. Several authors have demonstrated an additive stimulatory effect on inositol phosphate accumulation by two different stimulators, but the intracellular mechanism by which a cell recognizes two different inositol phosphates remains unknown. It has been demonstrated that, contrary to bombesin- or vasopressin-initiated inositol phosphate signaling, PDGF-stimulated inositol phosphate formation in 3T3 cells did not exhibit protein kinase C-mediated negative feedback control nor was it inhibited by pertussis toxin, which suggested the presence of multiple inositol phosphate signal pathways with different biochemical characteristics within a cell. Our results provide further evidence for the complexity of transmembrane signaling by phospholipid hydrolysis.

Acknowledgments

We thank Dr. M. P. Schrey, St. Mary’s Hospital Medical School, London, England, and our colleague Dr. E. F. Adams for their kind technical advice.

References


Manuscript received March 1, 1993. Accepted in final form September 14, 1993. Address reprint requests to: Tomoki Todo, M.D., Department of Neurosurgery, International Medical Center of Japan, 1–21–1 Toyama, Shinjuku-ku, Tokyo 162, Japan.