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Oligodendrocyte substratum adhesion modulates expression of adenylate cyclase-linked receptors

(myelinogenesis/2',3'-cyclic nucleotide 2'-phosphodiesterase/second messengers/myelin basic protein/phosphorylation)

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ABSTRACT **The molecular mechanisms of myelin formation/reformation in the central nervous system are unknown. In previous work we have demonstrated that mature oligodendrocytes (OLG) respond to a signal(s), elicited by their adhesion to a substratum, by turning on a myelinogenic metabolism. Events occurring within 24 hr of adhesion include generation of diacylglycerol, activation of protein kinase C, phosphorylation of myelin basic protein, and enhanced synthesis of myelin lipids and proteins. To elucidate the mechanism(s) of signal transduction, we have investigated whether OLG–substratum interaction influences the level of basal cAMP and the expression of receptors coupled to adenylate cyclase. By using ovine brain OLG we have found that adhesion to a polylysine-coated surface for 24 hr increased the basal level of cAMP 2-fold and altered the expression (assessed by cAMP production) of receptors coupled to adenylate cyclase. Isoproterenol (β-adrenergic agonist) augmented cAMP from 4 to 26 pmol/mg of protein in adhering OLG but had no such effect in nonattached OLG. Adhesion of OLG was accompanied by rapid synthesis of ethanolamine plasmalogen, a class of lipids believed to be associated with β-adrenergic receptors. Nonattached OLG responded to prostaglandin E_1 with only a 3-fold stimulation in their cAMP content; in attached OLG, 6-fold stimulation was observed. In contrast, vasoactive intestinal polypeptide elicited a 3-fold increase in cAMP in nonattached OLG but, following 24 hr of attachment, OLG did not respond to vasoactive intestinal polypeptide. The increase of cellular cAMP levels was accompanied by a 2.5-fold gain in protein kinase A. OLG–substratum adhesion resulted also in phosphorylation of the OLG/myelin protein, 2',3'-cyclic nucleotide 2'-phosphodiesterase, which proved to be a substrate for cAMP and phospholipid-, Ca^{2+}-dependent protein kinases. These findings, in conjunction with our earlier work, implicate cAMP and diacylglycerol in signaling myelinogenesis; they suggest that phosphorylation/dephosphorylation of myelin basic protein and 2',3'-cyclic nucleotide 2'-phosphodiesterase may be key processes in the cascade of events that are initiated by adhesion of OLG to a polylysine surface (possibly acting as a surrogate for axons) and culminate in the reformation of myelin.**

The signals that initiate and control *in situ* myelination by oligodendrocytes (OLG) are unknown. We have previously identified a substratum-induced signal in cultured OLG, presumably receptor mediated, that eventuates in (*i*) liberation of second messenger diacylglycerol and activation of protein kinase C with subsequent phosphorylation of myelin basic protein (MBP) (1); (*ii*) a rapid synthesis of myelin lipids (2) and proteins (3); and (*iii*) dramatic morphological changes (4, 5). Another second messenger, cAMP, has been implicated in the enhancement of myelinogenic properties of neonatal rat OLG (6). We have now examined the effect of OLG–substratum interaction (24 hr following adhesion) on cAMP levels and on the stimulation of receptors linked to adenylate cyclase by specific agonists. Herein we present evidence that subsequent to OLG–substratum adhesion there is (*i*) an overall increase in the level of cellular cAMP and cAMP-dependent protein kinase A activity; (*ii*) induction of β-adrenergic and prostaglandin E_1 (PGE_1) receptors and suppression of vasoactive intestinal polypeptide (VIP) receptors; and (*iii*) augmented synthesis of membrane plasmalogens. Furthermore, we show that the phosphorylation of 2',3'-cyclic nucleotide 2'-phosphodiesterase (CNPase) is adhesion dependent and, in contrast to MBP, is potentiated by cAMP as well as diacylglycerol.

MATERIALS AND METHODS

Isolation of OLG. OLG were isolated from 6-month-old lamb brains by a previously described procedure (7). Briefly, lamb brains kept at 4°C were scraped free of gray matter to obtain white matter. The white matter was finely minced and trypsinized [0.1% (wt/vol)] for 60 min at 37°C; ice-cold trypsin inhibitor [0.15% (wt/vol)] was added to arrest trypsin. Tissue was washed free of trypsin twice, suspended in 0.9 M sucrose, and homogenized by successive passage through screens of decreasing pore size (350 μm, twice; 210 μm; doubled 130 μm; doubled 52 μm, twice; 30 μm). Centrifugation at 850 × *g* for 10 min gave a floating layer of myelin and a cell pellet. The cell pellet was suspended in 0.9 M sucrose and layered over a 1.0–1.15 M sucrose gradient. After centrifugation at 431 × *g* for 12 min, three distinct bands were formed (bands 1–3 from top to bottom). Band 3 OLG (B3 OLG) were washed in Hanks' buffer, counted, and used for all of the experiments described.

Culture of OLG. B3 OLG were suspended in culture medium [Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% horse serum, 2 mM glutamine, 0.03 μg of fungizone per ml, and 2.4 μg of gentamicin sulfate per ml] at a density of ≈2.0 × 10^6 cells per ml and kept at 37°C and 5% CO_2 in air (8). Two culture conditions were used (8): (*i*) *nonattached*, for this B3 OLG were plated onto tissue culture plates, where they do not adhere; we refer to these cells as B3.f OLG (f = floating); (*ii*) *attached*, for this B3.f OLG after 3 days *in vitro* were transferred to polylysine-coated plates, where they adhere and following which they undergo changes in morphology and biology; we refer to these latter cells as B3.fA OLG (A = adherent). For the experiments described here, B3.f OLG after 3 days in culture were harvested, centrifuged at 200 × *g* for 3 min, and

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Abbreviations: OLG, oligodendrocyte(s); B3.f OLG, nonattached OLG; B3.fA OLG, attached OLG; MBP, myelin basic protein; CNPase, 2',3'-cyclic nucleotide 2'-phosphodiesterase; PGE_1, prostaglandin E_1; VIP, vasoactive intestinal polypeptide.

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divided into aliquots. One set of aliquots was replated onto tissue culture plates, where they continued as "floaters"; the other was plated onto polylysine-coated plates for attachment. Unless otherwise stated, all of the studies were performed on B3.f OLG and B3.fA OLG after 4 days *in vitro*—i.e., after 24 hr of adhesion for B3.fA OLG.

Measurement of cAMP levels. Drugs were prepared fresh at 100 times the desired final concentration and added in 10-μl aliquots to cultures in 1.0 ml of medium. All cultures received 3-isobutyl-1-methylxanthine (0.5 mM) to inhibit cAMP phosphodiesterase activity and allow cAMP synthesis to be studied independent of breakdown. After 30 min of incubation, the culture medium was aspirated and 0.2 ml of 6% ice-cold trichloroacetic acid was added to each well. The suspensions containing precipitated cells and free cAMP were collected in test tubes, sonicated briefly, and centrifuged at 1000 × *g* for 15 min. Protein content of the pellets was determined by the method of Lowry *et al.* (9). The supernatants were extracted with 2 ml of water-saturated ether four times, and the remaining aqueous phase was taken to dryness on an evaporator. Samples were then redissolved in distilled water, and cAMP levels were determined by radioimmunoassay with ^{125}I-labeled 2′-O-monosuccinyladenosine 3′,5′-cyclic monophosphate as a competitor for binding to anti-cAMP antiserum in 50 mM acetate buffer at pH 4.75 (10).

Determination of Plasmalogens. Cultures were labeled with 5 mCi (1 Ci = 37 GBq) of [U-^{14}C]glycerol in 1 ml of DMEM/horse serum for 24 hr prior to harvesting and isolation of Folch lower-phase lipids (11). Lipids were resolved by two-dimensional thin-layer chromatography with solvent systems 1 [chloroform/methanol/acetic acid/water, 65:25:8:8.4 (vol/vol)] and 2 [chloroform/methanol/ammonia/water, 92:36:2.8:3.2 (vol/vol)]. Ethanolamine and choline phospholipids were identified by iodine staining, scraped from the plates, and eluted from the silica gel with four washes of chloroform/methanol/water, 100:42:6 (vol/vol). The purified phospholipids were run again in the two-dimensional system except that the order of the first and second dimensions was reversed and the plate was exposed to HCl gas prior to development in the second dimension (12). Cleavage of the plasmalogen 1-vinyl ether bond by HCl gas results in the liberation of free aldehyde, which runs at the solvent front of the second dimension, and the 1-hydroxy 2-acyl phospholipid, which partitions less into the mobile phase and thus runs behind the diacyl phospholipid in the second dimension.

***In Vivo* Phosphorylation of CNPase.** OLG were labeled with $^{32}PO_4^{3-}$ (50 mCi/ml of Hepes-buffered phosphate-free DMEM) for a total of 40 min and drugs were added in 10-μl aliquots for the last 20 min. CNPase was immunoprecipitated from 100 μg of B3.f OLG or B3.fA OLG protein solubilized in buffer A (50 mM Tris·HCl/0.15 M NaCl/1% Triton X-100/0.04% $NaDodSO_4$, pH 7.4). Samples were incubated with antiserum (rabbit anti-bovine CNPase) (13) for 16 hr at 4°C with gentle rotation. Immune complexes were precipitated by the addition of protein A-Sepharose beads (Pharmacia); this was followed in 2 hr by centrifugation at 10,000 × *g* for 6 min. Immunoprecipitates were washed six times with buffer A, boiled for 1 min in sample buffer [3% $NaDodSO_4$/1 mM 1,4-dithio-L-threitol/60 mM Tris·HCl, pH 6.8/10% glycerol (wt/vol)/0.05% bromophenol blue (wt/vol)], and resolved by $NaDodSO_4$/polyacrylamide gel electrophoresis. Autoradiographs were obtained by exposing the dried gels to Kodak XK-5 film.

RESULTS

Freshly isolated OLG do not attach to tissue culture plates and exist as round, phase-bright cells floating in clusters. Transferring these B3.f OLG, after 4 days *in vitro*, to polylysine-coated plates results in their attachment (B3.fA OLG) and formation of a monolayer. During the first 24 hr after adherence there is little apparent change in morphology of these cells but their metabolism is significantly altered (2, 3). With time in culture, B3.fA OLG develop an intricate network of processes. We can maintain these cells in culture for 6 months and longer with a high degree of purity (>98%).

Influence of OLG–Substratum Adhesion on the Activation of Adenylate Cyclase-Linked Receptors. Equilibrium cAMP levels in B3.f OLG and B3.fA OLG were measured following 30-min incubations with various drugs in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine. Attachment of OLG to a polylysine substratum for 24 hr resulted in an increase of basal cAMP levels 2-fold over unattached controls (Fig. 1*A*). Forskolin and PGE_1 caused a 3-fold augmentation in basal cAMP levels in B3.f OLG,

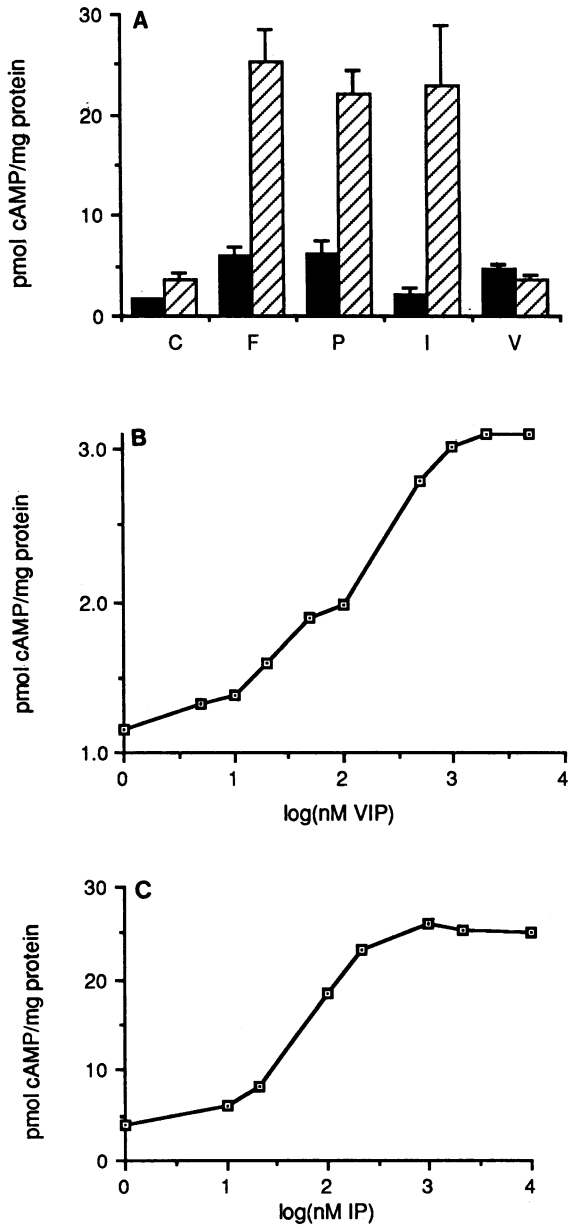

FIG. 1. Intracellular cAMP accumulation in B3.f and B3.fA OLG. (*A*) Response of B3.f (solid bars) and B3.fA OLG (hatched bars) to various compounds. C, water; F, forskolin (1 μM); P, PGE_1 (1 μM); I, isoproterenol (1 μM); V, VIP (1 μM). Results are reported as pmol of cAMP per mg of protein and are the means ± SEM of triplicate assays performed in a single experiment. The experiment was replicated three times. (*B*) B3.f OLG; dose–response curve for VIP. (*C*) B3.fA OLG; dose–response curve for isoproterenol (IP).

whereas in B3.fA OLG these compounds elicited a 6.5-fold increase. Surprisingly, isoproterenol, a prototypic β-adrenergic agonist, did not significantly elevate cAMP levels in B3.f OLG but produced a >6-fold elevation over basal levels in B3.fA OLG. The cAMP response to β-adrenergic agonists in B3.fA OLG was quantitatively similar to that of forskolin and PGE_1. Cellular levels of protein kinase A [measured by an *in vitro* transfer of phosphate to synapsin I (14)] increased 2.5-fold over the first 24 hr of attachment and remained constant thereafter (data not shown). In contrast, the concentration of protein kinase C was constant throughout (1).

The dose–response curves for VIP and isoproterenol-stimulated cAMP in B3.f OLG and B3.fA OLG, respectively, are shown in Fig. 1 *B* and *C*. Maximal stimulation occurred at ≈2 μM VIP and ≈1 μM isoproterenol. The EC_{50} for VIP in B3.f OLG was 200 nM and the EC_{50} for isoproterenol-stimulated cAMP responses in B3.fA OLG was 60 nM. These values indicate the presence of high-affinity adenylate cyclase-linked VIP receptors on B3.f OLG (but not B3.fA OLG) and of β-adrenergic receptors on B3.fA OLG (but not B3.f OLG) (15). None of the other receptors examined (see Table 1) has shown this adhesion-mediated modulation, although PGE_1 responses were greater in B3.fA OLG than in B3.f OLG.

B3.f OLG and B3.fA OLG are refractory to a number of ligands that act through known stimulatory or inhibitory receptors linked to adenylate cyclase (Table 1). The level of receptor-mediated cAMP generation in OLG appeared low relative to those reported in 2-day-old rat brain cultures (17, 18), rat astrocytes (16), or mouse neuroblastoma cell lines (10), even after the significant increase that accompanied the transition toward B3.fA OLG. These characteristics set OLG pharmacologically apart from astrocytes and neuroblastoma cell lines.

Synthesis of Plasmalogens by B3.f OLG and B3.fA OLG. Previously we have shown that significant increases in OLG/myelin-specific proteins and membrane lipids occur subsequent to OLG adhesion to substratum (2, 3). A hypothesis has been advanced that links increased plasmalogen metabolism to activation of β-adrenergic receptors (19). Most of the brain plasmalogens are localized in the OLG/myelin compartments. When the incorporation of [^{14}C]glycerol into plasmalogens was followed as a function of adhesion and over time in culture, we observed a 2- to 3-fold increase in the synthesis of plasmalogens, mainly ethanolamine plasmalogen (Fig. 2), coincident with adherence (i.e., in B3.fA OLG) and with augmented β-adrenergic receptor activity. However, a causal relationship between the two remains to be established. There was an increase in the synthesis of ethanolamine plasmalogens over time in culture, but this was small compared to the change that occurred upon OLG–substratum attachment (Fig. 2).

Table 1. Expression of adenylate cyclase-linked receptors by floating and adherent OLG compared to those expressed by adherent rat astrocytes and mouse neuroblastoma cells

Agonist	Ovine OLG B3.f	Ovine OLG B3.fA	Rat astrocytes*	Mouse neuroblastoma†
Dopamine	−	−	ND	+
Serotonin	−	−	−	+
VIP	+	−	+	ND
Secretin	−	−	+	+
Isoproterenol	±	++	+	−
PGE_1	+	++	+	+
DADLE‡	−	−	ND	+
SRIF‡	−	−	+	−

−, Absent; +, present; ++, increased expression with attachment; ND, not determined; DADLE, [D-Ala^2,D-Leu^5]enkephalin; SRIF, somatostatin.
*Ref. 16.
†Ref. 10.
‡Responses to DADLE and SRIF were determined in the presence of 1 μM PGE_1.

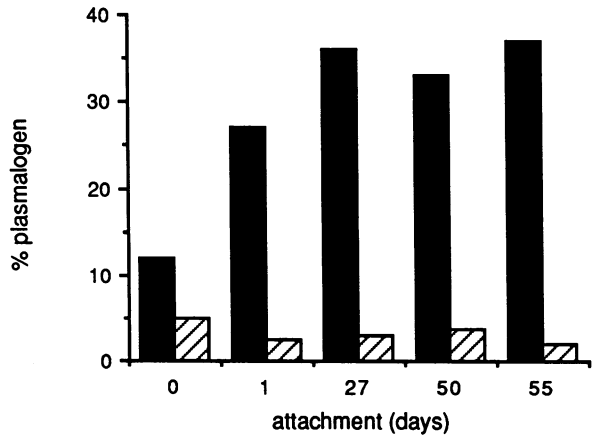

FIG. 2. Effect of OLG–substratum adhesion and time in culture on synthesis of plasmalogens by isolated OLG. Results are presented as % plasmalogen form for ethanolamine phospholipids (solid bars) and choline phospholipids (hatched bars). Note the significant increase in synthesis of ethanolamine plasmalogens within the first 24 hr of attachment; there is also a slight increase over time in culture. In contrast, synthesis of choline plasmalogens appears independent of attachment and time in culture.

Effect of Adhesion and Kinase Modifiers on the Phosphorylation of CNPase. Synthesis of CNPase is not influenced by OLG–substratum interaction (3). CNPase is phosphorylated *in situ* (20). Because phosphorylation of MBP is affected by adhesion, we investigated the role of substratum on the phosphorylation of CNPase. B3.f OLG and B3.fA OLG after 4 days in culture (1 day adherence for B3.fA OLG) were labeled with $^{32}PO_4^{3-}$ for 30 min prior to harvesting the cells and immunoprecipitating CNPase. Fig. 3*A* shows conclu-

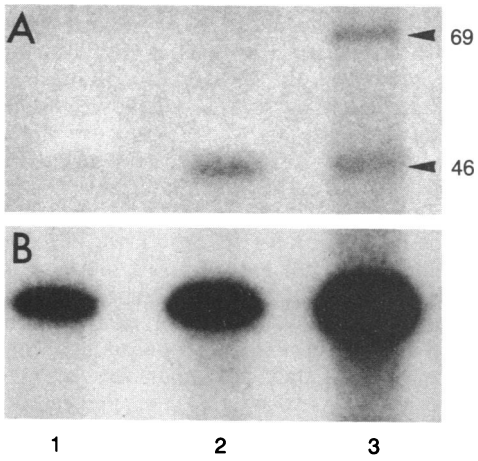

FIG. 3. Effect of OLG–substratum adhesion and drug treatment on CNPase phosphorylation. (*A*) Comparison of the extent of phosphorylation of CNPase in B3.f and B3.fA OLG cultures. Cultures of B3.f and B3.fA OLG after 4 days *in vitro* (1 day of adhesion for B3.fA) were labeled (see text). An autoradiograph is shown. Lane 1, B3.f OLG; lane 2, B3.fA OLG; lane 3, ^{14}C-labeled molecular weight standards. Numbers on the right are given as $M_r \times 10^{-3}$. Note that incorporation of $^{32}PO_4^{3-}$ into CNPase is only seen in the attached state—i.e., B3.fA OLG. (*B*) Effect of various drugs on $^{32}PO_4^{3-}$ incorporation into CNPase. Twenty-one-day-old B3.fA OLG were labeled and processed (see text). An autoradiograph is shown. Lane 1, control (0.1% ethanol in water); lane 2, forskolin (1 μM); and lane 3, phorbol 12-myristate 13-acetate (1 μM). Results shown are representative of four experiments.

sively that there was no CNPase phosphorylation by B3.f OLG, but the protein was phosphorylated by B3.fA OLG—i.e., adherence is a prerequisite for CNPase phosphorylation.

To investigate the type of kinases involved in CNPase phosphorylation, B3.fA OLG, after 21 days in culture, were exposed to drugs known to activate kinases. Forskolin, an activator of adenylate cyclase, stimulated the phosphorylation of CNPase as did phorbol 12-myristate 13-acetate, a positive effector of protein kinase C (Fig. 3*B*). These results show that CNPase is a substrate for protein kinases A and C. Protein kinases A and C phosphorylate the higher molecular weight (M_r 46,000) form of CNPase; serines are the only amino acids phosphorylated, although it is not yet known if the same residues are involved (unpublished data).

DISCUSSION

Many cell types respond to environmental signals by increased synthesis of second messengers. In this report, we have shown that generation of cAMP is stimulated by a signal(s) from OLG–substratum adhesion. There are quantitative and qualitative differences in cAMP regulation elicited by cell adhesion. Examples of the former are the responses of B3.f OLG and B3.fA OLG to forskolin and PGE_1 suggesting an adhesion-coupled elevation of adenylate cyclase and PGE_1 receptors, respectively, in B3.fA OLG. Examples of qualitative differences in the regulation of cAMP are the adhesion-related modulation of the response to isoproterenol (a β-adrenergic agonist) and VIP: B3.fA OLG only respond to isoproterenol, whereas B3.f OLG only respond to VIP.

We have previously postulated that upon adhesion to a substratum OLG metabolism undergoes a transition from myelin maintenance to myelin formation (2, 3, 5). Because B3.f OLG are in a mature state (i.e., derived from myelinated brains), this transition represents a regenerative process. Indeed, over time in culture, B3.fA OLG reform myelin (5, 21). Consequently, our results implicate a direct and a hormone-induced activation of the adenylate cyclase system in myelinogenesis, at least *in vitro*. Two questions appear pertinent. (*i*) Do these observations have physiological relevance? (*ii*) If so, what are the specific roles, if any, of β-adrenergic and VIP receptors during myelinogenesis, *in situ*?

β-Adrenergic and PGE_1 receptors have been detected in cultures of rat OLG (16, 17, 22) and in mouse oligodendroglioma (23). Induction of CNPase (6) and galactolipids (24) by exogenously added cAMP to cultures enriched in immature and mature rat OLG, respectively, has also been reported. The following facts speak in favor of a physiological significance for these receptors: (*i*) they are found in cells at very different stages of development (i.e., pre- and postmyelination OLG) and in different species; (*ii*) receptor activation appears to be implicated in induction of myelin components; and (*iii*) they are modulated by OLG–substratum adhesion, which also signals the synthesis of myelin components (3). It could be argued that our observations do not reflect a change in receptor number *per se*; rather, that they are a consequence of functional alterations of any or all of the components of the adenylate cyclase–receptor complex. Be that as it may, this should not detract from the fact that β-adrenergic and VIP receptors react differentially in B3.f OLG and B3.fA OLG, whatever the mechanism involved.

If β-adrenergic and PGE_1 receptors were to have a role in myelinogenesis, one must postulate an *in situ* exposure of OLG to catecholamines and prostaglandins. It has been suggested that prostaglandins are by-products of receptor-mediated breakdown of plasmalogens (19), a class of phospholipids that are enriched in OLG and myelin. The enhanced plasmalogen metabolism observed upon OLG adhesion to substratum may thus provide a source of prostaglandins that, in turn, may activate OLG through autocrine and/or paracrine mechanisms. A source for catecholamines in the central nervous system that may stimulate OLG has not been identified. However, β-adrenergic receptors have been linked to MBP methyl transferases (25); posttranslational methylation of MBP appears essential to normal myelin formation.

Two OLG/myelin proteins are known to be phosphorylated *in vivo*: MBP and CNPase (20). We have reported that substratum-induced phosphorylation of MBP is mediated by protein kinase C (1). We show here (Fig. 3*A*) that phosphorylation of CNPase is also adhesion dependent, whereas synthesis of CNPase is adhesion independent (3). Thus, as with MBP, phosphorylation of CNPase is effected by a signal (or signals) mediated by OLG–substratum interaction. However, phosphorylation of the two proteins seems to be regulated differently. Incorporation of $^{32}PO_4^{3-}$ into CNPase can be increased by activating either adenylate cyclase (forskolin) or protein kinase C (phorbol 12-myristate 13-acetate), indicating that CNPase is a substrate for protein kinases A and C (Fig. 3*B*). In contrast, in intact cells, MBP is only a substrate for protein kinase C. Moreover, elevating intracellular cAMP with forskolin results in a >3-fold decrease in phosphate incorporation into MBP (1). These observations suggest that second messengers, diacylglycerol and cAMP, may act concurrently in some instances (e.g., phosphorylation of CNPase) but oppose one another in others (e.g., phosphorylation of MBP) (1, 26). This type of interactive second messenger response is also observed in other cell types (27).

The role of VIP in OLG function and in the central nervous system at large is unknown, although VIP has been shown to enhance glycogenolysis in brain slices (28). Based on the fact that astrocytes are the accepted site of storage of brain glycogen, it was suggested that adenylate cyclase-linked VIP receptors on astrocytes (29) play a predominant role in central nervous system glycogenolytic responses. B3.f OLG respond to VIP, whereas B3.fA OLG do not. The loss of VIP responsiveness at a time when OLG undergo a significant elevation in metabolic activity toward synthesis of myelin components and the presence of VIP receptors in mouse oligodendroglioma (a cell line that expresses a very limited repertoire of OLG properties) (23) speak against a function for VIP in myelinogenesis and more likely suggest some role for VIP receptors in immature OLG.

In summary, we have demonstrated that, immediately following OLG adhesion to a polylysine substratum, there is (*i*) an overall elevation in basal cAMP and protein kinase A activity; (*ii*) the turning on of a response to isoproterenol; (*iii*) a heightened cAMP response to PGE_1 and forskolin; (*iv*) a loss of responsiveness to VIP; (*v*) phosphorylation of CNPase; and (*vi*) an increased synthesis of plasmalogens. Since adhesion of OLG to substratum also marks the beginning of a switch toward a myelinogenic metabolism (2, 3), it is suggested that these events may be coupled to one another.

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1. Vartanian, T., Szuchet, S., Dawson, G. & Campagnoni, A. T. (1986) *Science* **234**, 1395–1398.
2. Szuchet, S., Yim, S. H. & Monsma, S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7019–7023.
3. Yim, S. H., Szuchet, S. & Polak, P. (1986) *J. Biol. Chem.* **261**, 11808–11815.

4. Massa, P., Szuchet, S. & Mugnaini, E. (1984) *J. Neurosci.* **4,** 3128–3139.
5. Szuchet, S., Polak, P. E. & Yim, S. H. (1986) *Dev. Neurosci. (Basel)* **8,** 208–221.
6. McMorris, F. A. (1983) *J. Neurochem.* **41,** 506–515.
7. Szuchet, S., Arnason, B. G. W. & Polak, P. E. (1980) *J. Neurosci. Methods* **3,** 7–19.
8. Szuchet, S. & Yim, S. H. (1984) *J. Neurocsci.* **11,** 131–144.
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biochem.* **193,** 265–275.
10. Dawson, G., McLawhon, R. W. & Scheideler, M. A. (1982) in *Neuroscience Approached Through Cell Culture*, ed. Pfeiffer, S. E. (CRC, Boca Raton, FL), Vol. 2, pp. 89–114.
11. Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226,** 497–509.
12. Gunawan, J., Vierbuchen, M. & Debuch, H. (1979) *Hoppe-Seyler's Z. Physiol. Chem.* **360,** 971–978.
13. Sprinkle, T. J., Grimes, M. J. & Eller, A. G. (1980) *J. Neurochem.* **34,** 880–887.
14. Palfrey, H. C. & Mobley, P. L. (1987) in *Neurochemistry: A Practical Approach*, eds. Bachelard, H. & Turner, A. J. (IRL, Oxford), pp. 161–191.
15. Lefkowitz, R. J., Stadel, J. M. & Caron, M. (1983) *Annu. Rev. Biochem.* **52,** 159–186.
16. Evans, T., McCarthy, K. D. & Harden, T. K. (1984) *J. Neurochem.* **43,** 131–138.
17. McCarthy, K. D. & DeVellis, J. (1980) *J. Cell Biol.* **85,** 890–902.
18. Weingarten, D. P., Kumar, S., Bressler, J. & DeVellis, J. (1984) in *Oligodendroglia*, ed. Norton, W. T. (Plenum, New York), pp. 299–338.
19. Horrocks, L. A., Yeo, Y. K., Harder, H. W., Mozzi, R. & Goracci, G. (1986) in *Advances in Cyclic Nucleotide and Protein Phosphorylation Research*, eds. Greengard, P. & Robison, G. A. (Raven, New York), Vol. 20, pp. 263-292.
20. Bradbury, J. M., Campbell, R. S. & Thompson, R. J. (1984) *J. Biochem.* **221,** 351–359.
21. Szuchet, S. (1987) in *Glial Neuronal Communication in Development and Regeneration*, eds. Althaus, A. & Seifert, W. (Springer, Berlin), pp. 755–777.
22. Ventimiglia, R., Greene, M. I. & Geller, H. M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5073–5077.
23. Vartanian, T., Szuchet, S. & Dawson, G. (1985) *Trans. Am. Soc. Neurochem.* **16,** 259 (abstr.).
24. Pleasure, D., Parris, J., Stern, J., Grinspan, J. & Kim, S. U. (1986) *J. Neurochem.* **46,** 300–302.
25. Amur, S. G., Shanker, G. & Pieringer, R. A. (1986) *J. Neurosci. Res.* **16,** 377–386.
26. Ulmer, J. B., Edwards, A. M., McMorris, F. A. & Braun, P. E. (1987) *J. Biol. Chem.* **262,** 1748–1755.
27. Nishizuka, Y. (1984) *Science* **225,** 1365–1370.
28. Quach, T. T., Rose, C. & Schwartz, J. C. (1978) *J. Neurochem.* **30,** 1335–1341.
29. Rougon, G., Noble, M. & Mudge, A. W. (1983) *Nature (London)* **305,** 715–717.