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Full Paper

Serofendic Acid Promotes Stellation Induced by cAMP and cGMP Analogs in Cultured Cortical Astrocytes

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Abstract. We investigated the effect of serofendic acid, a neuroprotective substance derived from fetal calf serum, on the morphological changes in cultured cortical astrocytes. Cultured astrocytes developed a stellate morphology with several processes following exposure to dibutylyl cAMP (dbcAMP), a membrane-permeable cAMP analog; 8-Br-cGMP, a membrane-permeable cGMP analog; or phorbol-12-myristate-13-acetate (PMA), a protein kinase C activator. Serofendic acid significantly accelerated the stellation induced by dbcAMP- and 8-Br-cGMP. In contrast, the PMA-induced stellation was not affected by serofendic acid. Next, we attempted to elucidate the mechanism underlying the dbcAMP-induced stellation and explore the site of action of serofendic acid. Both the stellation induced by dbcAMP and the promotional effect of serofendic acid were partially inhibited by KT5720, a specific protein kinase A (PKA) inhibitor. Furthermore, serofendic acid failed to facilitate the stellation induced by Y-27632, an inhibitor of Rho-associated kinase (ROCK). These results indicate that serofendic acid promotes dbcAMP- and 8-Br-cGMP-induced stellation and the promotional effect on dbcAMP-induced stellation is mediated at least partly by the regulation of PKA activity and not by controlling ROCK activity.

Keywords: serofendic acid, astrocyte, stellation, cAMP, cGMP

Introduction

We previously identified a neuroprotective substance, serofendic acid, in the ether extract of fetal calf serum based on an ability to protect rat primary cortical neurons from nitric oxide (NO) donor–induced cytotoxicity (1). Serofendic acid was found to be a sulfur-containing atisane-type diterpenoid (15-hydroxy-17-methylsulfinyl-latisan-19-oic acid) (2). We also reported that the compound had a potent protective effect on the neurotoxicity induced by glutamate, nitric oxide, and oxidative stress without inhibiting glutamate receptors in cultured cortical, striatal, and spinal cord neurons (3 – 7). However, the effects of serofendic acid on cells in the central nervous system (CNS) other than neurons were not examined. Because serofendic acid was specifically present in fetal serum, and not adult serum (1), it is possible that it is closely associated with development and differentiation, accompanied by morphological changes of various cells.

Among the different types of cells present in the CNS, astrocytes are the most abundant in the brain and play many crucial roles in normal brain functions. For example, astrocytes maintain homeostasis in the extracellular fluid and form the blood–brain barrier (8). These roles are supported by the astrocyte's morphology with a wide surface area that is provided by multiple thin processes. In the developing CNS, morphological changes of astrocytes are observed. Immature astrocytes show a bipolar form, but they adopt a multipolar shape during the perinatal period. Finally, they undergo a progressive hypertrophy (9). Under pathological condi-

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tions such as ischemia and neurodegenerative diseases, reactive gliosis, which is characterized by dramatic changes in cell morphology and increased expression of immunoreactive glial fibrillary acidic protein (GFAP), occurs (10). Reactive astrocytes produce trophic factors that promote the survival of neurons and neurite outgrowth (11). On the other hand, the increased proliferation of astrocytes detected in brains following injury is considered a physical barrier to neuronal regeneration (12). Although the morphology is known to change depending on the extracellular environment, the significance of the morphological change of astrocytes remains to be completely understood.

Morphological changes are also observed in cultured astrocytes. Astrocytes generally display a flattened shape without processes, but a dramatic alteration in cell morphology, termed stellation, was caused by exposure to dbcAMP, a membrane-permeable cAMP analog, accompanied by rearrangements of actin (13, 14). Previous studies found that when cultures were incubated with 8-Br-cGMP, a membrane-permeable cGMP analog, pituicytes derived from the neurohypophysis adopted a stellate morphology with several processes (15). In astrocytes from the subfornical organ, treatment with an NO donor induced similar changes in the mediation of guanylate cyclase activation (16). Similar changes were observed in response to a change of pH (17); the presence of neurons (18); or exposure to Aβ (19), adenosine (20), a PKC activator (21), or a Rho kinase inhibitor (22). Thus, primary astrocyte cultures are widely used for elucidating the mechanism regulating cell morphology. The exploration of substances that can affect morphological changes is important for further understanding the mechanism and significance of these changes.

In the present study, we investigated the effect of serofendic acid on morphology in cultured cortical astrocytes with or without the reagents that have been previously reported to induce stellation. We demonstrated that serofendic acid acted as a positive regulator of the conversion of a flattened shape to a stellate shape. In addition, we demonstrated that dbcAMP provoked stellation partially via a PKA-mediated mechanism, not via an exchange protein directly activated by cAMP. We also attempted to identify the mechanism that underlies the promotional effect of serofendic acid on the stellation of astrocytes.

Materials and Methods

Materials

Eagle's minimum essential medium (Eagle's MEM) was purchased from Nissui Pharmaceutical (Tokyo). Fetal bovine serum was obtained from JRH Biosciences (Lenexa, KS, USA.). Ethanol, 10% formaldehyde neutral buffer solution, glucose, glutamine, HEPES, $NaHCO_3$, and paraformaldehyde were from Nacalai Tesque (Kyoto). 8-Bromo-guanosine-3',5'-cyclic monophosphate (8-Br-cGMP) and phorbol-12-myristate-13-acetate (PMA) were from Sigma (St. Louis, MO, USA). N^6,$O^{2'}$-Dibutyryl-adenosine-3',5'-cyclic monophosphate (dbcAMP), KT5720, and Mayer's hematoxylin solution were purchased from Wako (Osaka). Dispase and trypsin were from GIBCO, Invitrogen Japan (Tokyo). Eosin and ENTELLAN neu were obtained from Merk KGaA (Darmstadt, Germany). 8-(4-Chlorophenylthio)-2'-*O*-methyl-adenosine-3',5'-cyclic monophosphate (8-CPT-2-Me-cAMP) was from Tocris (Ellisville, MO, USA). Y-27632 was from Calbiochem (San Diego, CA, USA). N^6-Benzoyl-adenosine-3',5'-cyclic monophosphate (6-Bnz-cAMP) was from BIOLOG Life Science Institute (Bremen, Germany). Serofendic acid was synthesized according to procedures reported previously (2).

Cell cultures

Cortical type I astrocytes were prepared from postnatal day 1 pups of Wistar rats (Nihon SLC, Shizuoka) according to procedures described previously (23). Briefly, cells were dissociated from cerebral cortices of neonates by dispase, filtered through nylon mesh, and plated on uncoated 75-cm^2 flasks. Cultures were incubated in Eagle's MEM supplemented with 10% heat-inactivated fetal bovine serum, glutamate (2 mM), glucose (total 11 mM), $NaHCO_3$ (24 mM), and HEPES (10 mM) at 37°C in a humidified 5% CO_2 atmosphere. After the cells became confluent, non-astrocytes such as microglia were removed by shaking followed by changing the medium, and astrocytes were detached by trypsin and reseeded on 8-well chamber slides or 24-well plates. They were used for experiments at 1 – 2 weeks after the plating. The purity of astrocytes was >95% as determined by immunostaining with anti-glial fibrillary acidic protein antibody. The animals were treated in accordance with the guidelines of the Kyoto University animal experimentation committee and the guidelines of the Japanese Pharmacological Society.

Drug treatment

Cells seeded on 8-well chamber slides were washed with a serum-free medium and incubated in a serum-free medium supplemented with dbcAMP for 1 – 24 h or with 8-Br-cGMP, PMA, 8-CPT-2-Me-cAMP, 6-Bnz-cAMP, or Y-27632 for 3 h. Serofendic acid was simultaneously applied to the medium with these drugs. KT5720 was added 10 min before and during the exposure to these drugs.

Analysis of cell morphology

To reveal the morphological changes in cultured astrocytes, we used hematoxylin-eosin staining, which is a facile method compared with immunostaining, because the culture was confirmed to be highly pure. Cultures were fixed with 4% paraformaldehyde for 30 min, fixed with a 10% formaldehyde neutral buffer solution for 10 min, and stained with Mayer's hematoxylin solution for 7 – 10 min. After washing with water, cells were dyed again with 0.25% eosin–alcohol for 5 – 8 min and rinsed with 90% ethanol. Then, they were dehydrated, mounted with ENTELLAN neu, and observed using a microscope. Stellate astrocytes were defined as cells that have one or more processes longer than their cell bodies. The total number of cells and the number of stellate cells were counted, and the percentage of stellate cells was determined by counting >200 cells in multiple fields. To investigate morphological changes in detail, primary processes were counted. Astrocytes having no processes (0), a few processes (1 – 3), and many processes (=4) were defined as class I, II, and III, respectively; and the percentage of cell classes was calculated.

Statistics

Values were expressed as means ± S.E.M. The statistical significance of difference between groups was determined with Dunnett's test. Probability values less than 5% were considered to be significant.

Results

Stellation induced by dbcAMP, PMA, and 8-Br-cGMP in cultured cortical astrocytes

Cultured cortical astrocytes normally appeared in flat, polygonal shapes without processes (Fig. 1A). When cells were exposed to dbcAMP (1000 μM), they changed into stellate shapes bearing several processes (Fig. 1B). Exposure to dbcAMP (10 – 1000 μM) significantly induced morphological changes in a concentration-dependent manner (Fig. 2A). Treatment with dbcAMP for 3 – 6 h showed a maximal effect, followed by a gradual decline at 12 and 24 h (Fig. 2B). These results were consistent with a previous report (24). It is well recognized that morphological changes of astrocytes also occur in response to stimuli that cause an increase in the intracellular cGMP concentration or the activation of

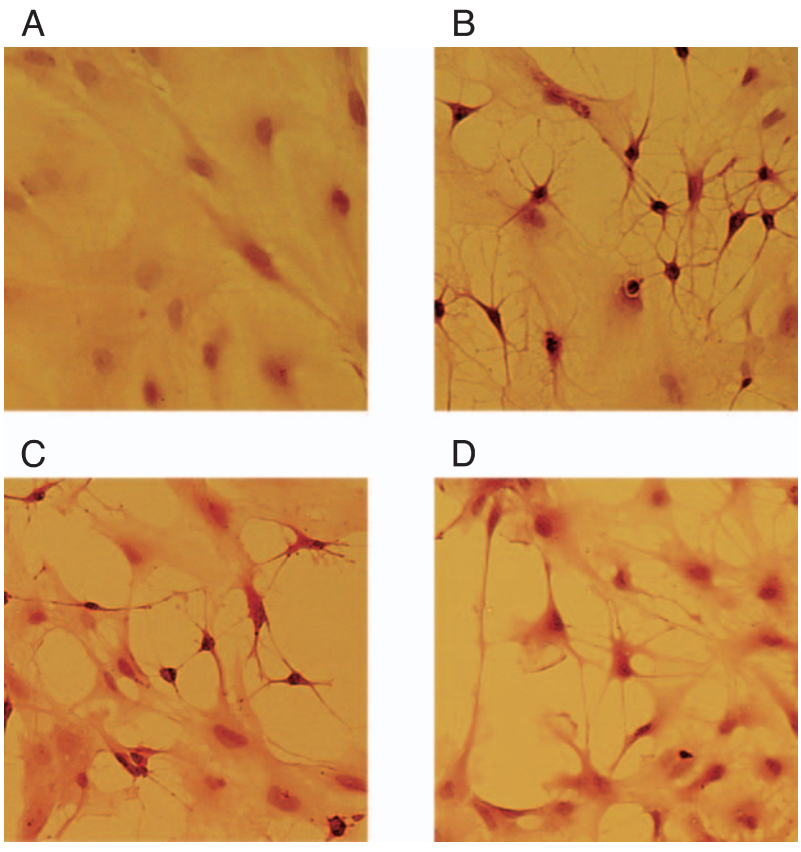

Fig. 1. Representative microphotographs of morphological changes in cultured cortical astrocytes. Cultures were stained with hematoxylin solution and eosin-alcohol. Cells were sham-treated (A) or treated with 1000 μM dbcAMP (B), 1000 μM 8-Br-cGMP (C), or 0.1 μM PMA (D) for 3 h. Calibration bar = 50 μm.

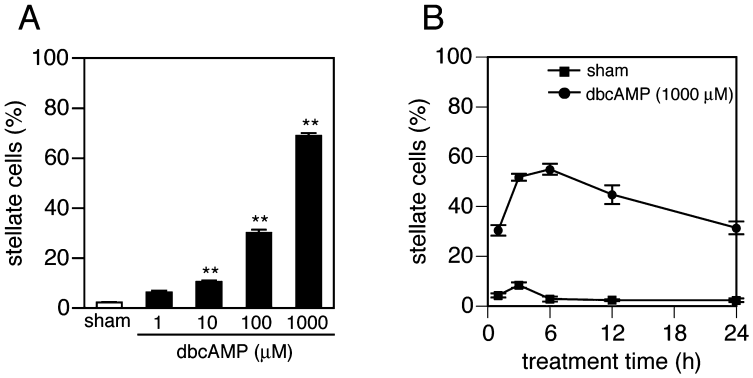

Fig. 2. Effect of dbcAMP on morphology in cultured cortical astrocytes. A: Concentration-dependent effects of dbcAMP on astrocyte morphology. Cells were exposed to dbcAMP (1 – 1000 μM) for 3 h. ***P*<0.01 *vs* sham. B: Time course of dbcAMP-induced stellation. Cells were treated with dbcAMP (1000 μM) for 1 – 24 h.

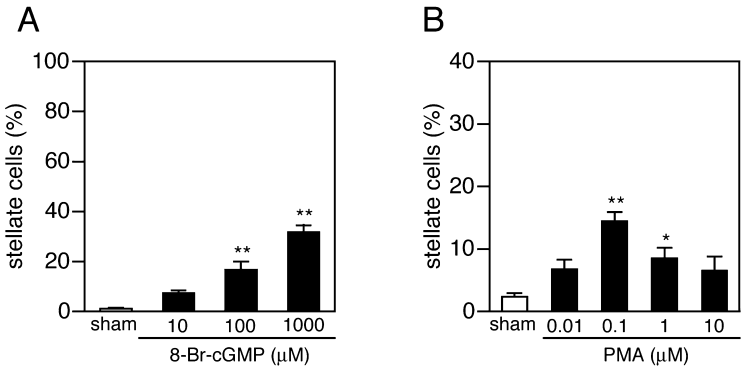

Fig. 3. Effects of 8-Br-cGMP (A) and PMA (B) on morphology in cultured cortical astrocytes. Cells were exposed to 8-Br-cGMP (10 – 1000 μM) or PMA (0.01 – 10 μM) for 3 h. **P*<0.05, ***P*<0.01 *vs* sham.

protein kinase C (PKC) (15, 21). Exposure of cultures to 8-Br-cGMP (100 – 1000 μM) evoked stellation in a concentration-dependent manner (Fig. 3A). Morphological changes were significantly caused by treatment of cultures with PMA, a PKC activator, at concentrations of 0.1 and 1 μM, although these changes were slightly weaker than the dbcAMP-induced stellation (Fig. 3B). Interestingly, while the dbcAMP and 8-Br-cGMP–induced stellation were similar in appearance, PMA-induced stellation was clearly distinguished from dbcAMP-induced stellation in the size of cell bodies and thickness of processes (Fig. 1).

Effect of serofendic acid on stellation in cultured cortical astrocytes

Next, we investigated whether the morphology of astrocytes was influenced by serofendic acid or not. Serofendic acid alone had no effect on cell morphology (Fig. 4). The coapplication of serofendic acid (1 – 100 μM) with dbcAMP (100 μM) for 3 h enhanced dbcAMP-induced stellation in a concentration-dependent manner (Fig. 5A). A significant facilitatory effect of serofendic acid was observed at 1 μM. Similarly, concurrent treatment of cultures with serofendic acid (100 μM) and 8-Br-cGMP (1 mM) significantly promoted 8-Br-cGMP–induced stellation (Fig. 5B). In contrast, PMA-induced stellation was not affected by the simultaneous administration of serofendic acid (100 μM) with PMA (0.1 μM) for 3 h (Fig. 5C).

To further understand the effect of serofendic acid on astrocyte morphology, we counted the number of primary processes of astrocytes and classified the cells into three groups according to the number of processes. Simultaneous administration of dbcAMP (100 μM) with serofendic acid (100 μM) induced stellation to the same extent as exposure to dbcAMP (1000 μM) alone (Table 1). However, treatment with dbcAMP (100 μM) and serofendic acid (100 μM) caused both a significant increase in the number of class III astrocytes and a decrease in the number of class II astrocytes compared with 1000 μM dbcAMP alone (Table 1). These results suggested that serofendic acid does not only enhance the stellation mediated by the cAMP-dependent pathway, but increases the number of primary processes via unknown mechanisms.

Involvement of protein kinase A (PKA) in dbcAMP-induced stellation

We focused on dbcAMP-induced stellation and attempted to elucidate the mechanism underlying it and explore the site of action of serofendic acid. We verified that serofendic acid also promoted stellation induced by isoproterenol, a β-adrenergic receptor agonist, which caused the activation of adenylate cyclase and increase

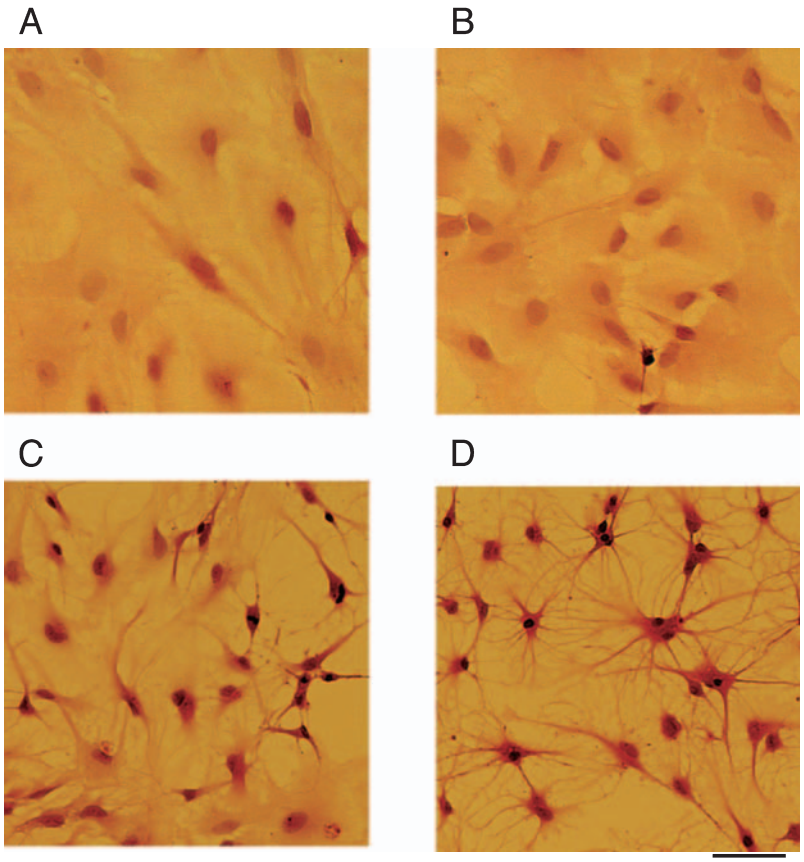

Fig. 4. Effect of serofendic acid on the dbcAMP-induced stellation in cultured cortical astrocytes. Cultures were stained with hematoxylin solution and eosin-alcohol. Mircrophotographs show sham (A)-, 100 μM serofendic acid (B)-, 100 μM dbcAMP (C)-, or 100 μM dbcAMP plus 100 μM serofendic acid (D)-treated cultures. Cells were exposed to dbcAMP (100 μM) for 3 h. Serofendic acid was simultaneously applied with dbcAMP for 3 h. Calibration bar = 50 μm.

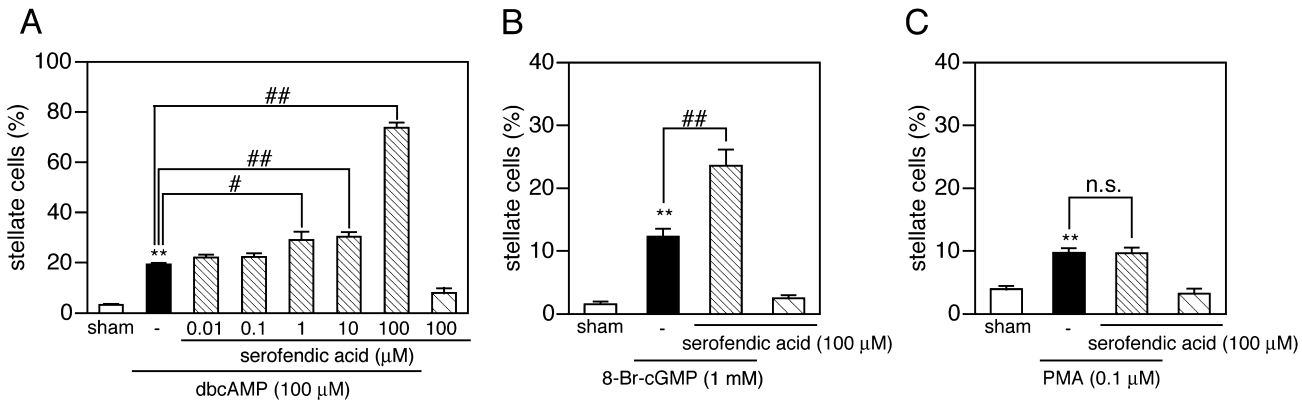

Fig. 5. Effects of serofendic acid on stellation induced by dbcAMP (A), 8-Br-cGMP (B), and PMA (C) in cultured cortical astrocytes. Cells were exposed to dbcAMP (100 μM), 8-Br-cGMP (1000 μM), or PMA (0.1 μM) for 3 h. Serofendic acid was simultaneously applied with these stimuli for 3 h. $^{**}P<0.01$ *vs* sham. $^{\#}P<0.05$, $^{\#\#}P<0.01$. n.s.: not significant.

of intracellular cAMP (data not shown). These results suggested that serofendic acid did not directly interact with dbcAMP.

PKA has been considered as representative of the target enzymes involved in various cellular responses elicited by cAMP. In order to confirm the involvement of PKA in dbcAMP-induced stellation and the promotion of stellation by serofendic acid, we investigated the

Table 1. Effect of serofendic acid on the number of primary processes in cultured cortical astrocytes

Treatment	% of total cells			
	class I (flattened cells)	class II	class III	class II + class III (stellate cells)
dbcAMP (1000 μM)	31.6 ± 2.6	34.9 ± 1.3	33.4 ± 1.4	68.4 ± 2.6
dbcAMP (100 μM) + serofendic acid (100 μM)	30.8 ± 1.0	25.4 ± 1.7**	43.7 ± 2.7$^{\#\#}$	69.2 ± 1.0

Cells were exposed to dbcAMP (100 – 1000 μM) for 3 h. Serofendic acid (100 μM) was simultaneously applied with dbcAMP for 3 h. $^{**}P<0.01$ and $^{\#\#}P<0.01$ *vs* % of class II and class III treated with dbcAMP (1000 μM), respectively.

effect of KT5720, a specific PKA inhibitor. When KT5720 (1 μM) was applied for 10 min before and 3 h during dbcAMP exposure, dbcAMP-induced stellation was partially suppressed (Fig. 6A). Also, the promotional effect of serofendic acid on dbcAMP-induced stellation was partially suppressed by KT5720 exposure (Fig. 6A).

Recently, a new family of cAMP-binding proteins, an exchange protein directly activated by cAMP (Epac), was identified (25, 26). To clarify the involvement of Epac in the astrocytic stellation, we attempted to compare the effects of a selective activator of PKA, 6-Bnz-cAMP, and a selective activator of Epac, 8-CPT-2-Me-cAMP, on astrocyte morphology (27). The morphology of cultured astrocytes was not affected by the addition of 8-CPT-2-Me-cAMP at 10 – 1000 μM (Fig. 6B), although dbcAMP significantly induced the stellation of cultured astrocytes at the same concentration. Incubation with 100 μM 6-Bnz-cAMP caused the occurrence of stellation in astrocytes. Moreover, 6-Bnz-cAMP–induced stellation was accelerated by 100 μM serofendic acid (Fig. 6C), indicating that dbcAMP induces stellation partially by the activation of PKA, not Epac, and serofendic acid also promotes stellation partly through the PKA-dependent pathway.

Effect of a ROCK inhibitor on the morphology in cultured astrocytes

The small GTP-binding protein Rho has been reported to be involved in the regulation of cell adhesion and morphology in various types of cells (28, 29). In astrocytes, botulinum C3 transferase, which inactivates the Rho GTPase, mimics cAMP-raising agents in inducing stellation (30). Stellation was also caused by the inhibition of Rho-associated kinase (ROCK), one of the downstream targets of Rho (22). These studies suggest that the Rho/ROCK pathway plays a critical role in determining the morphology of astrocytes. Therefore, we examined the effect of serofendic acid on stellation induced by a selective ROCK inhibitor, Y27632. Application of Y27632 (1 – 100 μM) induced stellation in a concentration-dependent manner (Fig. 7A). The stellation induced by Y-27632 showed no obvious difference from that triggered by dbcAMP. However,

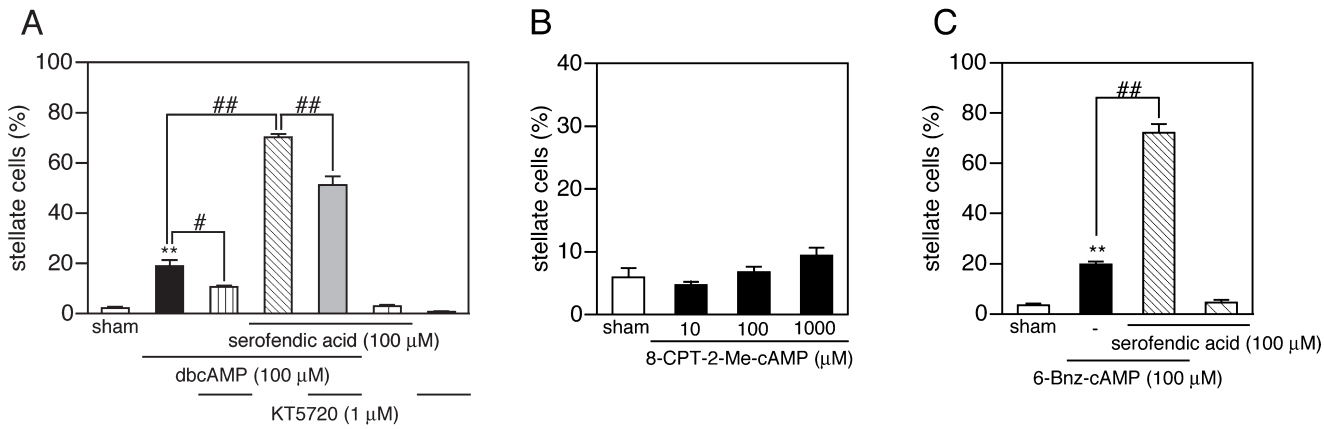

Fig. 6. Involvement of PKA and Epac in dbcAMP-induced stellation in cultured cortical astrocytes. A: Effect of KT5720 on the stellation induced by dbcAMP with or without serofendic acid. Cells were exposed to dbcAMP (100 μM) and/or serofendic acid (100 μM) for 3 h. KT5720 (1 μM) was added 10 min before and during the exposure to dbcAMP. $^{**}P<0.01$ *vs* sham. $^{\#}P<0.05$, $^{\#\#}P<0.01$. B: Effect of 8-CPT-2-Me-cAMP on astrocyte morphology. Cells were exposed to 8-CPT-2-Me-cAMP (10 – 1000 μM) for 3 h. C: Effect of serofendic acid on 6-Bnz-cAMP–induced stellation. Cells were exposed to 6-Bnz cAMP (100 μM) and/or serofendic acid (100 μM) for 3 h. $^{**}P<0.01$ *vs* sham. $^{\#\#}P<0.01$.

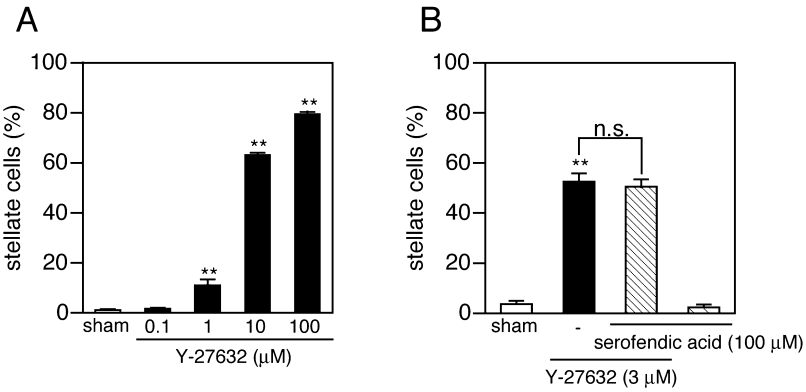

Fig. 7. Effect of serofendic acid on Y-27632–induced stellation in cultured cortical astrocytes. A: Concentration-dependency of the effects of Y-27632 on astrocyte morphology. Cells were exposed to Y-27632 (0.1 – 100 μM) for 3 h. $^{**}P<0.01$ *vs* sham. B: Effect of serofendic acid on Y-27632–induced stellation. Cells were exposed to Y-27632 (3 μM) for 3 h. Serofendic acid was simultaneously applied with Y-27632 for 3 h. $^{**}P<0.01$ *vs* sham. n.s.: not significant.

the stellation evoked by Y-27632 was not affected by the simultaneous administration of 3 μM Y-27632 with 100 μM serofendic acid (Fig. 7B), indicating that serofendic acid does not act on the inactivation of ROCK in promoting stellation in cultured astrocytes.

Discussion

In the present study, we demonstrated that serofendic acid promoted stellation induced by the activation of a cAMP- and cGMP-dependent pathway in cultured cortical astrocytes. However, the physiological role and importance of astrocytic stellation are largely unclear. In the developing CNS, immature astrocytes, which exhibit a bipolar shape, differentiate into multipolar astrocytes bearing radial processes, resembling stellate astrocytes treated with dbcAMP or 8-Br-cAMP. Furthermore, it has been reported that the expression of a glial glutamate transporter, GLT-1, is weak in both embryonic and neonatal brains and strong in mature brains (31). GLT-1 protein levels increased when mesencephalic astroglial cultures were maintained with dbcAMP (32). Thus, stellate astrocytes were assumed to be an in vitro model of differentiated astrocytes.

Concomitant incubation with serofendic acid and dbcAMP resulted in a marked enhancement of the change in morphology of cultured astrocyte induced by dbcAMP alone, whereas the shape of astrocytes was not affected by serofendic acid alone. Furthermore, Serofendic acid increased the number of primary processes of the astrocytes, which stellated to the same extent as dbcAMP alone. These results indicate that serofendic acid may exert various effects depending on the degree of cell differentiation in the brain. Under pathological conditions such as ischemia and Alzheimer's disease, astrocytes become reactive and start to proliferate to form glial scars, which are considered a hindrance to axonal regeneration. Since reactive astrocytes and stellate astrocytes are characterized by the increased expression of GFAP, some researchers believe that stellate astrocytes correspond to reactive astrocytes. In contrast, several studies suggest that cAMP is a neurotrophic mediator in the central nervous system acting on cells such as retinal ganglionic cells and cerebellar granule cells (33, 34). Increasing levels of cGMP also had an anti-apoptotic effect in various cells such as neurons and astrocytes (35, 36). In addition, recent reports have demonstrated that cAMP-elevating agents are neuroprotective of dopaminergeic neurons, reducing the proliferation of deleterious astrocytes (37). Collectively, these findings have led us to the possibility that elevating the level of intracellular cAMP and cGMP could exert beneficial effects on various cells, and the potentiation of cAMP- and cGMP-induced stellation by serofendic acid also serves a protective function by inhibiting the hyperprolifelation of astrocytes.

Both cAMP and cGMP are typical second messengers that mediate the intracellular signaling by neurotransmitters and hormones, and these signal pathways have been reported to interfere with each other at several target proteins (25). For example, in platelets, while increased cGMP levels lead to activation of PKG-I followed by a suppression of platelet aggregation, they elevate intracellular cAMP levels through inhibition of phosphodiesterase 3, and consequent platelet activation (38). We determined that the stellation induced by both dbcAMP and 8-Br-cGMP was promoted by serofendic acid and showed a similar appearance. These results suggest that the stellation is mediated by a common signaling pathway, which is also regulated by serofendic acid. Preliminary data have shown that KT5720, a PKA inhibitor, does not block 8-Br-cGMP–induced stellation (data not shown). Accordingly, PKA does not seem to be a common signaling pathway of astrocytic stellation induced by cAMP and cGMP. Moreover, serofendic acid increased the number of primary process induced by dbcAMP, but not by 8-Br-cGMP (data not shown). These results suggested that cAMP and cGMP induced

the stellation via independent pathways. Accordingly, these signals may affect the stellation additively or synergistically. In contrast, the coapplication of serofendic acid with PMA had no effect on PMA-induced stellation. In comparison with astrocytes treated with dbcAMP, PMA elicited astrocytes bearing long thick processes and extended cell soma. These reagents may induce stellation through the mediation by two separate signals, consistent with previous results (21).

We demonstrated that dbcAMP caused astrocytic stellation partially via a PKA-dependent pathway. Since dbcAMP-induced stellation was not completely inhibited by treatment with KT5720, it is suggested that a PKA-independent pathway to stellate astrocytes exists. It is well known that besides PKA, cAMP can activate at least two different signal transduction pathways. One of them is direct stimulation of Epac followed by activation of GTPase Rap-1 (26). Stellation induced by dbcAMP, however, may not be mediated by the activation of Epac, as shown by the inability of 8-CPT-2-Me-cAMP to induce morphological change in astrocytes. Another pathway acts on a guanine nucleotide exchange protein, called CNrasGEF, which directly activates Ras (39). Previous reports revealed that CNrasGEF is expressed predominantly in the brain, with a widespread distribution, and activated by both cAMP and cGMP analogs. Therefore, CNrasGEF may be associated with dbcAMP- and 8-Br-cGMP–induced stellation in addition to PKA.

Stellation is accompanied by a reduction in the number of focal adhesion sites and a loss of stress fibers (30). In cultured astrocytes, dihydrocytochalasin B, which directly disrupts cortical actin filaments, also caused a retraction of cytoplasm and elongation of processes similar to those seen with dbcAMP (14). In addition, ROCK directly or indirectly phosphorylates myosin light-chain in various cells, thereby increasing the binding to actin filaments and subsequently the formation of stress fibers (40). These findings indicate that the rearrangement of the actin cytoskeleton occurs downstream of the inactivation of ROCK. Furthermore, the pathway linking PKA to the inactivation of Rho in cultured astrocytes is still unknown, but in other cells, this pathway seems to be clear. For example, PKA phosphorylates active Rho on residue Ser188 and thereby inhibits Rho downstream signaling in human natural killer cells (41). In the present study, Y-27632-induced stellation was not affected by serofendic acid, suggesting that the promotional effect of serofendic acid is not due to the regulation of ROCK activity. Thus, the regulatory effect of serofendic acid on stellation may occur upstream of ROCK, probably through the control of PKA activation.

We previously reported that the concentration of serofendic acid contained in fetal calf serum was in the order of tens of nM (1). In addition, serofendic acid significantly prevented acute glutamate neurotoxicity at a concentration as low as 1 nM (3). In the present study, the concentration of serofendic acid required to promote morphological change was higher than the order of μM. One possible reason for the requirement of this high concentration is that astrocytes in vivo are not completely mimicked by those in cultures in terms of the responsiveness to various stimuli. To our knowledge, this is the first report concerning a promotional effect on astrocytic stellation by natural products. Moreover, stellate astrocytes treated with dbcAMP and serofendic acid had more radial processes and an increased number of branches compared to those treated with dbcAMP alone. This finding indicates that serofendic acid is distinct from many of the reagents known to induce stellation and have additive effects on stellation. Thus, the morphological features induced by serofendic acid are new and have not been reported previously.

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