Effect of Dibutyryl Cyclic AMP-Treatment on Prostaglandin F$_{2\alpha}$-Stimulated Phosphoinositide Hydrolysis in Cultured Rat Astrocytes

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ABSTRACT—Dibutyryl cyclic AMP (dBCAMP)-treatment of cultured rat astrocytes induced changes in astrocyte morphology followed by the potentiation of prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$)-stimulated phosphoinositide (PI) hydrolysis. The potentiation was observed in astrocytes of the cerebral cortex, cerebellum, and hippocampus. The dBCAMP-treatment induced agonists-specific changes in PI hydrolysis; e.g., the potentiation of norepinephrine-effect and the reduction of the carbachol-effect. Coincubations of carbachol or norepinephrine with PGF$_{2\alpha}$ produced additive responses.

Cultured astrocytes alter their polygonal-shaped morphology into a process-bearing one in response to various agents, such as, dibutyryl cyclic AMP (dBCAMP), phorbol esters, and hormones (1–4). It is due to the reorganization of the cytoskeletal proteins including glial fibrillary acidic protein (GFAP) (3–6). The changes in astrocyte morphology are accompanied by the modulations of the important cellular functions (7, 8). We recently reported that prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) stimulates phosphoinositide (PI) hydrolysis in cultured rat astrocytes via its putative receptors and that dBCAMP modulates the response (9). This communication describes the further characteristics of the effects of dBCAMP-treatment on PI hydrolysis elicited by PGF$_{2\alpha}$ in cultured rat astrocytes.

Postnatal astrocytes from the cerebral cortex, cerebellum and hippocampus were prepared from 1-day-old Sprague-Dawley rats as described previously (9). The culture medium was Eagle’s minimum essential medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 10% fetal bovine serum (Hazleton) and 2 mM L-glutamine and was exchanged twice a week. Cell cultures were maintained in a humidified atmosphere of 95% air and 5% CO$_2$ at 37°C and grown to confluence in two weeks. Then, the cells were differentiated in the culture medium containing 1 mM dBCAMP (Sigma Chemical Co.) for a further four to six days unless otherwise indicated. At this stage, more than 90% of the cells from all three regions were GFAP-positive, and the morphology was stellate and of a process-bearing type. Cells were then loaded with [³H]-myo-inositol (Amersham, 18.3 Ci/mmol, 1 μCi/ml). Measurement of PI hydrolysis was carried out as described previously (9) and expressed as % total incorporated counts. The total radioactivities of [³H]-myo-inositol-prelabeled phospholipids per protein contents were not statistically different between the dBCAMP-treatment group and the control one of three brain regions. PGF$_{2\alpha}$

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(Funakoshi Pharmaceuticals, Tokyo) was used in a 0.1% ethanol solution.

Of the astrocytes that were not treated with dBcAMP (1 mM) (control group), cerebral cortical astrocytes showed a dose-dependent response (PI hydrolysis) to PGF$_2\alpha$ (Fig. 1A), as reported previously (9). In hippocampal astrocytes, the basal activity of PI hydrolysis (11.3 ± 0.9%) was relatively high as compared with those of astrocytes from both the cerebral cortex (4.7 ± 1.1%) and cerebellum (5.2 ± 1.3%), and PGF$_2\alpha$ at 1 μM significantly stimulated the PI hydrolysis. In contrast, PGF$_2\alpha$ at up to 1 μM had little effect on PI hydrolysis in cerebellar astrocytes (Fig. 1A).

After the cells were treated with dBcAMP (1 mM) for four to six days prior to the addition of PGF$_2\alpha$, the astrocytes of the three regions showed dose-dependent responses (Fig. 1B). In cerebral cortical astrocytes, the maximal response was about two-fold that of the control cells. In hippocampal astrocytes, dBcAMP-treatment reduced the basal activity of PI hydrolysis (7.2 ± 2.5%) as compared with that of the control cells. The values of PGF$_2\alpha$-elicited PI hydrolysis at 1 μM in hippocampal astrocytes were 9.1% in control cells (Fig. 1A) and 16.3% in dBcAMP-treated ones (Fig. 1B). It should be noted that in cerebellar astrocytes, cell differentiation caused a significant induction of PGF$_2\alpha$-stimulated PI hydrolysis, which suggests a possible expression of PGF$_2\alpha$ receptors by dBcAMP-treatment in cerebellar astrocytes. Incubation of astrocytes with dBcAMP (1 mM) for 2 hr in a serum-free medium caused the rapid astrocytic morphological change of polygonal types to stellate ones without affecting the response to

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**Fig. 1.** Dose-response curves for prostaglandin (PG) F$_2\alpha$-stimulated PI hydrolysis in cultured rat astrocytes from cerebral cortex (●), cerebellum (○) and hippocampus (▲). Cells were treated without (A) or with (B) dBcAMP (1 mM) for four to six days prior to the addition of 1 μCi/well of [3H]inositol for 24 hr. Cells were then washed three times with lithium-Krebs-Ringer buffer and incubated for 20 min, and subjected to PGF$_2\alpha$ for 15 min at the indicated concentrations. Values of % hydrolysis at time 0 were 4.0 ± 0.3, 4.3 ± 0.5 and 5.0 ± 0.5 for cerebral cortical, cerebellar and hippocampal astrocytes, respectively (control group, A) and 5.8 ± 0.7, 4.7 ± 0.7 and 10.5 ± 1.4 for cerebral cortical, cerebellar and hippocampal astrocytes, respectively (1 mM dBcAMP-treated group, B). Values are means ± S.E.M. of three experiments. *P < 0.05, **P < 0.01, ***P < 0.001, compared with none.
PGF$_2\alpha$ (29.1 ± 2.1% and 28.0 ± 3.1% elicited by 1 μM PGF$_2\alpha$ in the control and dBcAMP-treated cells, respectively). This means that the potentiation of the PGF$_2\alpha$-response is not due to the apparent morphological transformation. In rat C6 glioma cells, PGF$_2\alpha$ did not stimulate PI hydrolysis (data not shown).

Astrocytes have been shown to have cholinergic and adrenergic receptors on their cell surfaces (10). We therefore examined the effect of dBcAMP-treatment on carbachol- and norepinephrine-stimulated PI hydrolysis in rat cerebral cortical astrocytes. In both non-transformed and transformed astrocytes, carbachol and norepinephrine evoked PI hydrolysis in dose-dependent manners with maximal responses at 0.1 mM and 10 μM, respectively, and with ED$_{50}$ values of 13 μM and 0.5 μM, respectively. DBcAMP-treatment significantly increased the response to norepinephrine as well as to PGF$_2\alpha$ (Fig. 2, A and B). In contrast, the cholinergic response was reduced by the dBcAMP-treatment. This suggests that there are changes in the densities of cholinergic and adrenergic receptors during dBcAMP-treatment. When cells were co-incubated with the maximal doses of PGF$_2\alpha$ and carbachol or norepinephrine, additive responses were observed in both non-transformed and transformed astrocytes (Fig. 2), indicating that PGF$_2\alpha$ acts on its specific receptors.

In conclusion, dBcAMP-treatment increased PGF$_2\alpha$-stimulated PI hydrolysis in cultured rat astrocytes from all three regions. Regarding the regional difference in the PGF$_2\alpha$-response after differentiation by dBcAMP, as described in the method, the dBcAMP-treatment equally differentiated the cells from all regions, which were based on the morphological observations. It is therefore suggested that the expression of PGF$_2\alpha$ receptors after differentiation has regional specificity. PGF$_2\alpha$ exists in mammalian brains (11) and acts on the central nervous system, producing physiological activities (12). The present results as well as our previous study (9) predict possible roles of PGF$_2\alpha$ receptors in glial cell functions.

**Fig. 2.** Effects of dBcAMP-treatment on agonists-stimulated PI hydrolysis in rat cerebral cortical astrocytes. Cells were treated without (A) or with (B) 1 mM dBcAMP for four to six days and then used for the experiments. The concentrations used were: carbachol (CCh), 0.1 mM; norepinephrine (NE), 10 μM; PGF$_2\alpha$, 1 μM. Values are means ± S.E.M. of four to six experiments. □: Control, □: PGF$_2\alpha$. 

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