Protein kinase C regulates neurite outgrowth in spinal cord neurons

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Abstract: Objective The functional roles of protein kinase C (PKC) in the neurite outgrowth and nerve regeneration remain controversial. The present study was aimed to investigate the role of PKC in neurite outgrowth, by studying their regulatory effects on neurite elongation in spinal cord neurons *in vitro*. **Methods** The anterior-horn neurons of spinal cord from embryonic day 14 (E14) Sprague-Dawley (SD) rats were dissociated, purified and cultured in the serum-containing medium. The ratio of membrane-PKC (mPKC) activity to cytoplasm-PKC (cPKC) activity (m/c-PKC) was studied at different time points during culture. **Results** Between 3-11 d of culture, the change of m/c-PKC activity ratio and PKC- β II expression in the neurite were both significantly correlated with neurite outgrowth ($r=0.95$, $P<0.01$; $r=0.73$, $P<0.01$, respectively). Moreover, PMA, an activator of PKC, induced a dramatic elevation in the m/c-PKC activity ratio, accompanied with the increase in neurite length ($r=0.99$, $P<0.01$). In contrast, GF 109203X, an inhibitor of PKC, significantly inhibited neurite elongation, which could not be reversed by PMA. **Conclusion** PKC activity may be important in regulating neurite outgrowth in spinal cord neurons, and β II isoform of PKC probably plays a major role in this process.

Keywords: protein kinase C; spinal cord neurons; neurite outgrowth; *in vitro*

1 Introduction

Protein kinase C (PKC) is a family of Ca^{2+} - and phospholipid-dependent serine/threonine kinases that are involved in different cell signaling pathways. PKC is usually classified into 3 groups based on their structural and biochemical features: classical PKCs (α , β I, β II, and γ), new PKCs (δ , ϵ , η , θ , and μ), and atypical PKCs (ζ , λ , and τ). These isozymes differ not only in the cellular and subcellular distributions, but also in the ability to phosphorylate certain substrates, indicating that they have different functions and are differentially regulated^[7,30]. Being critical in inositol phospholipid

metabolism, PKC enzymes phosphorylate many proteins and regulate physiological effects, such as neuron activity, signal transduction, neurotransmitter release, synaptic plasticity, cell proliferation and gene expression^[20,21,23]. The role of PKC in the development and regeneration of the nervous system has also been widely investigated. PKC isoforms are implicated in the promotion of axonal regeneration, based on the increase in their expression in regenerating neurites and axons^[16,23,25,34,36]. For example, PKC isozymes α , β and γ are all up-regulated in their expression and translocated into the growth cones of regenerating sciatic nerve axons^[23]. This rapid increase in axonal PKC expression occurs within hours after nerve injury and has been shown to co-stimulate axonal regeneration with other factors^[23]. In contrast, inhibition of PKC has been found to inhibit the regenerative axonal growth^[3,5,6,11,34,35]. Also, PKC enzymes are important sig-

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nalizing molecules in preventing neurodegeneration after the nervous system injury. Activators of PKC, such as phorbol esters and bryostatin 1, can enhance neuronal survival and induce neurite outgrowth in a PKC- β I-dependent manner^[17]. The classical PKC could result in a contrast growth response of the central nervous system (CNS) neurons to myelin-associated inhibitors^[31]. GAP-43, a growth-associated protein, acts as an indicator of axon outgrowth when phosphorylated by PKC. In addition, PKC can phosphorylate the signal transducer and activator of transcription 3 (STAT3) at Ser727, which is phosphorylated primarily by Janus kinase (JAK) at Tyr705^[32]. These findings indicate that PKC enzymes are implicated in nerve regeneration after nerve injury. However, the role of PKC in neurite growth and regeneration remains controversial. Recent studies show that classical PKCs appear to mediate the inhibitory effects of myelin and chondroitin sulfate proteoglycans (CSPGs) on axonal regeneration^[29], while PKC inhibitors or kinase-dead mutants can reverse the inhibitory effects and promote axonal regeneration and neurite growth^[13]. To further elucidate the functional roles of PKC in neurite outgrowth and nerve regeneration, the expression of PKC in cultured spinal cord neurons was investigated in the present study, and the correlation between neurite outgrowth and PKC activity was also studied by using the PKC activator phorbol 12-myristate 13-acetate (PMA), or the inhibitor GF 109203X.

2 Materials and methods

2.1 Cell culture Culture medium, seruma, putrecine, trypsin (1:250), metrizamide, polylysine, histone III, phosphatidylserine (PS), diacylglycerol (DAG), leupeptin, PMSF, PMA, GF 109203X and diaminobenzidine (DAB) were all from Sigma. Laminin was from Gibco BRL. Monoclonal antibody anti-Neurofilament-200 (NF-200) was purchased from Department of Pathophysiology of Chongqing Medical University, China. Antibodies against PKC- α , - β I, - β II, and - ϵ , as well as Triton X-100 and SP reagent kits, were all from Beijing zhongshan Biotechnology CO., LTD, China. [γ -³²P]-ATP was provided by Biological Engineer Company of Beijing Yahui, China. The L15 culture medium contained 0.63 mg/mL sodium bicarbonate, 5 μ g/mL insulin, 0.1 mmol/L putrescine, 20 nmol/

L progesterone, 20 nmol/L glucose, 100 IU/mL penicillin, 100 μ g/mL streptomycin, 10 % fetal calf serum (FCS) and 10 % horse serum (HS).

2.2 Primary culture of spinal cord neurons The cell culture method of Camu^[4] was modified to improve the yield of neurons by using 5-FU to inhibit glial cell mitosis instead of using immuno-panning. Spinal cords were dissected from embryonic day 14 (E14) Sprague-Dawley (SD) rats, free of meninges and chopped into 1-2-mm³ pieces. After digestion with 0.125 % trypsin in D-Hanks for 20 min at 37 °C, L15 culture medium containing serum was added to stop the digestion, and tissue pieces were dissociated by gentle trituration. After being washed with culture medium containing 4 % BSA, cells were resuspended in 1 mL L15 culture medium and then carefully added onto the top surface of pre-equilibrated metrizamide (w/v: 6.8 % in L15-bicarbonate) in a 5-mL centrifuge tube. After centrifugation at 500 g for 15 min, the sharp band on the top of the metrizamide cushion was collected, washed and re-suspended in L15 culture medium. Then the cells were plated on poly-Lysine and laminin pre-coated cover slips at a concentration of 4.5×10^5 /L in 24-well culture plates. Cells were incubated at 37 °C with 95% O₂ and 5% CO₂. After 48-h culture, 12.5 μ g/mL 5-FU was added to the medium to inhibit glia mitosis. After 24 h, the medium was replaced by fresh medium without 5-FU. After that, the culture medium was replaced every 3 d. Neurons were cultured for 3, 5, 7, 9 and 11 d before being fixed in 4 % paraformaldehyde for immunohistochemical or biochemical analysis.

2.3 Neuron identification and image analysis Cultured neurons were fixed in 4% paraformaldehyde for 30 min. The cells were washed thoroughly with PBS and permeabilized with 0.1% Triton X-100 in PBS for 30 min. After that, the cells were incubated in blocking solution containing 5% BAS and 2 % goat serum prepared in PBS for 1 h at room temperature, followed by incubation with monoclonal mouse anti-NF-200 (1:100) overnight at 4 °C. After being washed, the cells were incubated with biotinylated goat anti-mouse IgG (1:200) for 3 h. Then the cells were incubated with horseradish peroxidase streptavidin for 3 h at room temperature and visualized in 0.01 mol/L Tris-HCl-buffered saline (pH 7.6) containing 0.05 % 3,3'-DAB and 0.01 % H₂O₂ for 10 min. Finally, the cells

were dehydrated with serial concentrations of ethanol solution, cleared with xylene and sealed with neutral gum. As a negative control, cells were incubated with PBS instead of the primary antibody used above. The percentage of NF-200-positive cells was calculated.

The neurite length of cultured neurons was analyzed with the Image Quantitative Analysis System (provided by Chongqing University, China). The longest neurite of the cultured neuron was scored. Ten microscopic fields containing at least 30 cells were randomly selected and scored in each of the duplicate plates from at least 3 separate experiments.

2.4 Immunohistochemistry for PKC- α , - β I, - β II or - ϵ and image analysis Fixed neurons were stained for PKC- α , - β I, - β II or - ϵ as described above, with polyclonal antibodies (1:100). The average optical densities of each protein in cell bodies and neurites were analyzed with Image Quantitative Analysis System.

2.5 Drug treatment to regulate PKC activity After 3-d culture, neurons were treated with PKC activator PMA (1×10^{-10} , 1×10^{-9} , 1×10^{-8} , 1×10^{-7} , and 1×10^{-6} mol/L) for 48 h or 96 h. Then the cells were fixed for NF-200 immunohistochemical staining and the longest neurite was measured in different groups. According to the results, the neurons were treated with 1 nmol/L PMA, 1 μ mol/L PKC inhibitor GF 109203X, or first with GF 109203X at 1 μ mol/L for 30 min and then PMA at 1 nmol/L. In the control group, cells were treated with neither PKC activator nor PKC inhibitor. After 96-h treatment, PKC activity and neurite length were analyzed.

2.6 PKC extraction Neurons in different groups were lysed by ultrasonic treatment in 2 mL enzyme extract buffer (containing 20 mmol/L Tris-HCl, 250 mmol/L sucrose, 2 mmol/L PMSE, 2 mmol/L EDTA, 2 mmol/L DTT, 10 mmol/L EGTA, and 0.01 % leupeptin), and centrifuged at 300 g for 15 min at 4 °C. The supernatant was collected and further centrifuged at 100 000 g for 1 h at 4 °C to harvest cytoplasmic PKC (cPKC). For membrane PKC (mPKC), the pellet was re-suspended in 2 mL enzyme extract buffer (containing 5 % Triton X-100) and mixed vigorously. After being incubated at 4 °C for 1 h, the mixture was centrifuged at 100 000 g for 1 h at 4 °C, and the supernatant was collected. Protein quantity was determined

using Lowry method^[19].

2.7 Measurement of PKC activity PKC activity was measured as previously described^[19]. The reaction mixture was composed of 2.5 mmol/L Tris-HCl (pH 7.5), 10 mmol/L MgCl₂, 0.5 mmol/L CaCl₂, 40 μ g histone III, 20 μ g PS, 5 μ g DAG, 25 μ L enzyme sample, and γ -³²P-ATP 37 KBq in a final volume of 200 μ L. The mixture was incubated at 37 °C for 5 min and the reaction was stopped by adding 2 mL pre-colded 15 % trichloric acetic acid. The precipitant was collected on micro-hole filter membranes. The membranes were washed twice with 15 % trichloric acetic acid, dried completely and then put in scintillation vials. After equilibrium for 4 h in scintillation solvent (dimethylbenzene + popo + popop) in darkroom, radioactive parameters of samples were counted automatically by LKB-1217 liquid flick automatic counting instrument made in Finland, and PKC activities were calculated according to the following formula:

PKC activity (pmol/min per mg protein) =

$$\frac{(\text{reaction tube cpm} - \text{control tube cpm}) \times 100}{\text{total counted cpm} \times \text{protein quantity in sample}}$$

The total counted cpm was 1 μ L γ -³²P-ATP dropping film and counting. The change of m/c-PKC activity ratio indicates the change of PKC activity.

2.8 Statistical analysis Experiments were performed in at least duplicate and each experiment was repeated at least 3 times. Data were expressed as mean $\pm$ SEM and analyzed by analysis of variance (ANOVA) followed by Newman-Keuls' test.

3 Results

3.1 Growth properties of primary cultured spinal cord neurons At 4 h of cell culture, the neurons began to adhere to the cover slips. And 24 h later, 90 % cells completed the adhesion and some neurons extended processes. After 48-72 h, neurons were growing in a healthy state. The cell bodies were clear and bright, and the neurite extended quickly (Fig. 1A). On day 7 of cell culture, the neurite length reached a peak, and the processes were somewhat thick (Fig. 1B). After

Fig. 1 Time course of neuron growth. **A:** On day 3 of cell culture, neurons were growing in a good state and neurite extended quickly. **B:** On day 7, the cell body area and neurite length reached a peak. **C:** On day 9, the neurite started to shrink. **D:** Purity of spinal cord neurons was confirmed by NF-200 immunostaining. **E:** Quantification of neurite length and cell body area by image analysis ($n=100$). $^{**}P < 0.01$. Scale bar: 20 μm .

7 d, the neurites stopped growing and began to shrink (Fig. 1C). The NF-200 immunohistochemical staining results showed that the purity of neurons reached above 85% (Fig. 1D). The statistical results of the neurite length and the body area were indicated in Fig. 1E.

3.2 Changes of PKC activity during cell culture The expression of PKC- α , - βI , - βII and - ϵ was analyzed by immunohistochemical staining (Fig. 2A-D). In neurites, expression of all the PKC isoforms increased from cultured day 3. The expression of PKC- α reached a peak on cultured day 5, and then declined, while the expression of other 3 PKC isoforms reached a peak on cultured day 7 and declined thereafter (Fig. 2E). Among these isoforms, only the expression of PKC- βII in neurites significantly coincided with the processes ex-

tension curve ($r = 0.73$, $P < 0.01$), while the relative coefficients between the expression of α , βI , or ϵ in the neurites and neurite length were 0.09, 0.63, and 0.65, respectively ($P > 0.05$). In the cell body, expression of all the PKC isoforms also increased from cultured day 3. The expression of PKC- βII reached a peak on cultured day 5, and then declined, while the expression of other 3 PKC isoforms reached a peak on cultured day 7 and declined thereafter (Fig. 2F).

Besides, in spinal cord neurons, PKC activity changed with the cultural time, accompanied with the neurite elongation. The value of m/c-PKC increased in the first 7 d, and then declined (Fig. 2G). Moreover, this trend of change in PKC activity was significantly correlated with the neurite outgrowth ($r = 0.95$, $P < 0.01$).

Fig. 2 Changes of PKC activity. The expression of (A) PKC- α , (B) - β I, (C) - β II and (D) - ϵ in cell bodies and neurite was analyzed by immunocytochemistry method. E: In neurites, expression of all the PKC isoforms increased from day 3. The expression of PKC- α reached a peak on day 5, and then declined, while the other 3 PKC isoforms reached a peak on day 7 and then declined. F: In cell bodies, the expression of all the isoforms began to increase on cultured day 3. The expression of PKC- β II reached a peak on cultured day 5 while expression of the other 3 subtypes peaked on day 7. G: The value of m/c-PKC increased gradually in the first 7 d and then declined, accompanied by neurite elongation. $n = 100$ in A, B, C and D. $n = 5$ in G. Scale bar: 20 μ m.

3.3 Regulation of neurite outgrowth by PKC activity The neurite outgrowth could be accelerated by treatment with PMA at concentrations of 1×10^{-10} , 1×10^{-9} , 1×10^{-8} , 1×10^{-7} , and 1×10^{-6} mol/L for 48 h or 96 h, among which 1×10^{-9} mol/L (1 nmol/L) PMA treatment for 96 h produced the optimal effect (Fig. 3). Thus, treatment with 1 nmol/L PMA for 96 h was used in the following experiments. After 96-h treatment of 1 nmol/L PMA, the length of processes was 30 % longer than that of the control group ($P < 0.05$). In contrast, treatment with 1 μ mol/L GF 109203X could inhibit the neurite outgrowth, and the average length of neurites was remarkably shorter

Fig. 3 Increased PKC activity could promote neurite outgrowth. PMA stimulation promoted neurite outgrowth at different concentrations compared with the control. The length of neurite treated with 1 nmol/L PMA for 96 h was longer than that of other groups. ** $P < 0.01$ vs other groups.

Fig. 4 PKC regulates neurite outgrowth. **A:** After 96-h treatment with PMA, the length of processes was 30% longer than that of the control group ($P < 0.05$). However, treatment with GF 109203X lead to the remarkable shortening of neurite length, compared to that in the control group ($P < 0.05$). Moreover, the neurite outgrowth of cells with the first GF 109203X treatment for 30 min followed by PMA treatment, was not significantly different from that with GF109203X treatment alone. **B:** After the treatment with PMA, m/c-PKC activity ratio increased dramatically ($P < 0.01$), while the treatment with GF 109203X induced a significant decrease in m/c-PKC activity ratio ($P < 0.01$). Similarly, there was no significant difference between GF 109203X treatment for 30 min followed by PMA treatment, and GF109203X treatment alone. * $P < 0.05$, † $P < 0.01$ vs GF 109203X treatment only or GF 109203X plus PMA treatment. † $P < 0.05$, ** $P < 0.01$ vs Control.

than that of the control ($P < 0.05$). Besides, the effect of 30-min treatment of 1 $\mu\text{mol/L}$ GF 109203x could not be significantly reversed by the following PMA treatment (Fig. 4A). Moreover, all the neurons were dead after application of 2 $\mu\text{mol/L}$ GF109203X (data not shown).

3.4 PKC activity regulated by chemicals After treatment with 1 nmol/L PMA, m/c-PKC activity ratio increased dramatically, compared with that of control group ($P < 0.01$). In the contrast, treatment with 1 $\mu\text{mol/L}$ GF 109203X induced a significant decrease in m/c-PKC activity ratio ($P < 0.01$). However, treatment with 1 nmol/L PMA afterward could not significantly reverse the effect of 30-min treatment with GF 109203X ($P > 0.05$) (Fig. 4B).

4 Discussion

Recently, many researchers have reported that PKC isoforms are implicated in promoting axonal regeneration, since studies indicate that their expression is increased in regenerating neurites and axons^[16,23,25,34,36]. The classical PKC can result in a contrasting growth response of CNS neurons to myelin-associated inhibitors^[31]. In addition, PKC can phosphorylate STAT3 at Ser727, which is phosphorylated primarily by JAK at Tyr705^[32]. In the contrast, inhibition of PKC has been found to decrease the axonal growth^[3,5,6,11,34,35]. These findings indicate that PKC participates in nerve regeneration after nerve injury. However, He's group^[29] have provided bio-

chemical and functional evidence that classical isoforms of PKC are key components in the signaling pathways that mediate the inhibitory activities of myelin components and chondroitin sulfate proteoglycans (CSPGs). Pharmacological or genetical block of PKC activity can attenuate the abilities of CNS myelin and CSPGs to activate Rho, thus inhibiting neurite outgrowth, suggesting that PKC mediates myelin- and CSPG-induced neurite outgrowth inhibition. Thus, the actual role of PKC in nerve regeneration remains still unclear. In the present study, we demonstrate that PKC activity might be important in regulating neurite outgrowth in spinal neurons, and βII isoform of PKC probably plays a major role in this process.

From Fig. 2, we observed a remarkable change in PKC activity during the period of neurite outgrowth. After activation, the PKC enzyme is translocated from cytoplasm to membrane. Thus, the ratio of mPKC activity to cPKC activity was used to indicate the PKC activity since only mPKC can trigger signal transduction. We find that the length of neurite increased, accompanied with the increase in m/c-PKC ratio during the first 7 d of culture, and then the neurite stopped growing and some began to shrink thereafter. Coincidentally, m/c-PKC ratio also declined during this period, and the changing trend was significantly correlated with that of neurite outgrowth ($r = 0.95$, $P < 0.01$). This finding suggests that PKC may regulate neurite outgrowth of cultured

neurons. Interestingly, the expression of isoforms α , β I, β II and ϵ reached a peak in cultured day 5 or 7 *in vitro*, and then declined. On cultured day 7, isoforms β I and β II were mainly expressed in neurite, suggesting that these isoforms might be more relevant to neurite outgrowth than α or ϵ isoform. Our findings are consistent with that of Miki^[20,21] who find that PKCs are present in mature and developing axons. In mature axons, only PKC- β is evidently expressed in the cytoskeletal elements, while in the developing axons, PKC- α , - β , and - ϵ are evidently distributed throughout the axoplasm. It is possible that each subspecies of PKC is involved in different aspects of axogenesis such as axonal elongation, pathfinding, synaptogenesis, and morphological and functional maturation in the growing motor axons. Being a critical component of intracellular signal transduction system, PKC can catalyze phosphorylation of many intracellular proteins and participate in many physiological processes. The supporting role of PKC to neurite outgrowth can be attributed to the ability of PKC to catalyze the phosphorylation of growth-associated protein (GAP-43)^[14,32]. GAP-43, a protein involved in the process of neuronal plasticity, is a well studied phosphoprotein that appears to be involved in nerve regeneration. Phosphorylated GAP-43 is known to promote neurite extension. In contrast, no evidence suggests that GAP-43 serves as the substrate for PKA or PKG. In addition, PKC can phosphorylate STAT3 at Ser727, which is phosphorylated primarily by JAK at Tyr705^[32]. Therefore, the inhibition of PKC alone should be sufficient to change the phosphorylation state of this phosphoprotein^[34]. Studies demonstrate that different isozymes phosphorylate the same proteins to different extents. Previous studies^[8,14,28] show that GAP-43 is more readily phosphorylated by PKC- β than by PKC- α or - ϵ . Here we investigated the expression of isoforms α , β I, β II and ϵ , and found that only expression of PKC- β II in neurites was significantly correlated with the processes extension curve ($r = 0.73$, $P < 0.01$). Within growth cones, GAP-43 is associated with membrane skeleton and hence the substrate serves to generate and maintain cell morphology^[1,8,14]. However, only phosphorylated GAP-43 can regulate the plasticity and the growth of growth cone^[1,2,8,14,22,38]. Under the same experimental conditions, we also investigated the ex-

pression of Ca^{2+} -independent PKC- ϵ during neurite outgrowth. Before day 5, PKC- ϵ expression increased slowly. From day 5, its expression increased rapidly, reaching a peak on day 7 and declined. This pattern is also coincident with that of neurite outgrowth. This finding demonstrates that the maintenance of growth cone activities may require both Ca^{2+} -dependent and Ca^{2+} -independent PKCs^[16].

Specific PKC activators and inhibitors are useful tools to study PKC functions. In the present study, we used PMA and GF 109203X to examine the roles of PKC in neurite growth. The ratio of m/c-PKC activity is highly correlated with the activity of neurite growth. As has been expected, after treatment with PMA, the ratio of m/c-PKC activity increased in the cultured spinal neurons while treatment with GF 109203X induced a significant decrease (Fig. 4). Besides, studies of Wu^[35] show that the PKC activator PMA could significantly enhance neurite outgrowth whereas the PKC inhibitor GF 109203X inhibits it. Our data are consistent with this result. Moreover, our data show that PMA treatment afterward cannot reverse the inhibitory effect of GF 109203X on neurite growth and PKC activity, suggesting that GF 109203X binds PKC irreversibly. These data also suggest that neurite outgrowth is sensitively affected by PKC activity.

However, the mode of PKC action in spinal cord neurite outgrowth is still unknown. It is possible that the function of PKC on neurite growth depends on the extracellular environment. Under the inhibitory environment such as in the injured CNS^[13,29], PKC regulates the inhibitory effects on neurite growth by acting on the upstream of RhoA, leading to neurite degeneration. Under the permissive environment, PKC may positively regulate neurite growth by activating the regeneration-related kinases, leading to nerve outgrowth. Besides, some evidence indicates the possibility that PKC acts on the upstream of focal adhesion kinase (FAK)^[10,18,27], latter of which is an essential kinase for the integrin signaling pathway required for cell adhesion-promoted neurite outgrowth on laminin^[15]. PKC activity is required for both integrin-mediated cell spreading and FAK phosphorylation^[9]. Since our preparations were cultured on laminin which promotes neurite outgrowth of spinal cord neurons, it is possible that FAK also plays a role in enhanced neuritogenesis

in the spinal cord neurons.

In conclusion, our results demonstrate that PKC is involved in the control of neurite outgrowth of cultured spinal cord neurons. Also, PKC, particularly the β II isozyme, is closely linked to the processes extension of cultured neurons. This would be useful to design strategies for nerve regeneration by targeting PKC pathways. Future studies are needed to elucidate the signaling cascade and to determine whether the regulation by PKC is relevant to the *in vivo* enhancement of nerve regeneration by specific drugs.

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蛋白激酶 C 调节脊髓神经元突起的生长

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摘要: **目的** 关于蛋白激酶 C (PKC) 在神经元突起生长和神经再生中的作用, 目前仍存有争议。本研究主要观察 PKC 对离体培养的脊髓神经元生长的调节作用, 旨在阐明 PKC 对突起生长的调节作用。**方法** 分离纯化胎龄 14 天 (E14) 的 SD 胎鼠的脊髓前角神经元, 进行原代培养, 并检测不同时相点膜/浆 PKC 活性 (m/c-PKC activity) 的比值。**结果** 神经元培养 3-11 d 期间, 神经元内 m/c-PKC 比值以及 PKC- β II 在突起中的表达水平均与突起生长呈显著相关关系 ($r = 0.95, P < 0.01$; $r = 0.73, P < 0.01$)。此外, PKC 激动剂 PMA 能显著提高 m/c-PKC 比值, 且与神经突起的生长一致 ($r = 0.99, P < 0.01$)。而 PKC 抑制剂 GF 109203X 则能显著抑制突起生长, 且不被 PMA 作用所逆转。**结论** PKC 的活性在脊髓神经元突起生长调节中具有重要作用, 其中 β II 亚型可能扮演重要角色。

关键词: 蛋白激酶 C; 脊髓神经元; 突起生长; 离体