

## Protein Kinase C $\gamma$ (PKC $\gamma$ ): Function of Neuron Specific Isotype

Naoaki Saito<sup>1</sup> and Yasuhito Shirai

Laboratory of Molecular Pharmacology, Biosignal Research Center, Kobe University, Kobe 657-8501

Received October 9, 2002; accepted October 9, 2002

The gamma isotype of protein kinase C (PKC $\gamma$ ) is a member of the classical PKC (cPKC) subfamily which is activated by Ca<sup>2+</sup> and diacylglycerol in the presence of phosphatidylserine. Physiologically, PKC $\gamma$  is activated by a mechanism coupled with receptor-mediated breakdown of inositol phospholipid as other cPKC isotypes such as PKC $\alpha$  and PKC $\beta$ . PKC $\gamma$  is expressed solely in the brain and spinal cord and its localization is restricted to neurons, while PKC $\alpha$  and PKC $\beta$  are expressed in many tissues in addition to the brain. Within the brain, PKC $\gamma$  is the most abundant in the cerebellum, hippocampus and cerebral cortex, where the existence of neuronal plasticity has been demonstrated. Pharmacological and electrophysiological studies have shown that several neuronal functions, including long term potentiation (LTP) and long term depression (LTD), specifically require PKC $\gamma$ . Generation of mice deficient in PKC $\gamma$  provided more information regarding the physiological functions of this isotype. PKC $\gamma$  deficient mice (i) have modified long term potentiation (LTP) in hippocampus, (ii) exhibit mild deficits in spatial and contextual learning (iii) exhibit impaired motor coordination due to persistent multiple innervations of climbing fibers on Purkinje cells, (iv) show attenuation of opioid receptor activation, and (v) show decreased effects of ethanol on type A of  $\gamma$ -aminobutyric acid (GABA) receptor. Furthermore, a point mutation in the PKC $\gamma$  gene may contribute to retinitis pigmentosa and Parkinsonian syndrome. This article reviews the specific functions of this neuron-specific isotype of PKC in neuronal signal transduction.

**Key words:** GABA receptor, knockout mice, long term depression, long term potentiation, Parkinson disease.

### Overview

PKC $\gamma$  is a member of the classical PKCs (cPKC) which was first isolated as one of more than 10 PKC cDNAs from a brain cDNA library (1). The cPKCs, PKC $\alpha$ , PKC $\beta$ , and PKC $\gamma$ , are activated by diacylglycerol (DAG) and Ca<sup>2+</sup> in the presence of phosphatidylserine (2, 3). PKC $\gamma$  was separated from PKC $\alpha$  and PKC $\beta$  biochemically using hydroxyapatite column chromatography, and the enzymological properties of the three isotypes were compared (4). Enzymological properties of this neuron specific isozyme are similar to those of PKC $\alpha$  and PKC $\beta$  which are described elsewhere in this series of reviews. The elucidation of PKC $\gamma$ -specific function was first approached by determining the localization of PKC $\gamma$  within the central nervous system. PKC $\gamma$  shows a unique neuronal distribution and intracellular localization in the brain (5–7) but the involvement of this PKC isotype in the specific neuronal function is still unclear. The generation of mice deficient in PKC $\gamma$  in 1993 (8, 9) provided an invaluable tool for the detailed analysis of PKC $\gamma$ -specific function. Although the appearance and behavior of the PKC $\gamma$ -knockout mice are not obviously abnormal, the experiments testing fine physiological

and behavioral responses revealed significant effects of deletion of this enzyme. In this review, we focus on the physiological functions of this isozyme and its involvement in etiology of diseases.

### 1. Genomic and protein structure

The cDNA for PKC $\gamma$  was sequenced in 1986 with those of PKC $\alpha$  and PKC $\beta$  (10, 11), then the genomic structure of PKC $\gamma$  and its chromosomal mapping were analysed. The human and mouse PKC $\gamma$  genes are localized on chromosomes 17q13.4 and 7, respectively. The human PKC $\gamma$  gene is found on the most distal part of the chromosome, suggesting that there might be a telomeric position effect modifying the gene's expression throughout the replicative lifespan of human cells. The PKC $\gamma$  gene is approximately 24.4 kb long and composed of small 18 exons varying between 32 and 406 bp in size (12). The AUG translation initiation site for open reading frames of PKC $\gamma$  is localized in exon 1 as other cPKCs. Deletions or translocations involving the chromosomal region of PKC $\gamma$  are frequently associated with malignant diseases such as leukemia [The Cancer Genome anatomy Project: recurrent chromosome aberrations in cancer (<http://cgap.nci.nih.gov/Chromosomes/RecurrentAberrations>)], although the functional role of this neuron specific PKC isotype in cancer is unclear. The 5'-flanking region of mouse PKC $\gamma$  gene lacks TATA and CAAT boxes but contains the binding sites of transcription factors, including AP2 and SP1 (13). The region responsible for neuron-specific expression of PKC $\gamma$  remains to be clarified.

<sup>1</sup>To whom correspondence should be addressed. Tel: +81-78-803-5961, Fax: +81-78-803-5961, E-mail: naosaito@kobe-u.ac.jp

Abbreviations: PKC, protein kinase C; LTP, long term potentiation; LTD, long term depression; DAG, diacylglycerol; GABA,  $\gamma$ -aminobutyric acid; RP, retinitis pigmentosa; mGluR1, metabotropic receptor 1; PLC $\beta$ 4, phospholipase C $\beta$ 4.

PKC $\gamma$  has C1 and C2 domains which bind DAG and Ca<sup>2+</sup>, respectively (14, 15). Both second messengers are necessary for the activation of cPKCs and fatty acids or lysoPC further enhances the activity of cPKCs (16, 17). The C1 domain of PKC $\gamma$  consists of two cystein-rich repeats (C1A and C1B), both of which bind DAG with high affinity (18, 19); all nPKC family members have a single high affinity DAG binding site in C1B region. The structure of the PKC $\alpha$ , PKC $\beta$ , and PKC $\gamma$  C1 domains are similar, while the amino acid sequences of their C2 domains show quite low homology. The functional differences resulting from this low homology are not known, but PKC $\gamma$  appears to have higher affinity to Ca<sup>2+</sup> than the others in the presence of phosphatidylserine (20).

## 2. Localization

The neuron specific distribution of PKC $\gamma$  is the most unique characteristics of this isotype. PKC $\gamma$  mRNA is solely found in the brain and spinal cord; it has not been found in any other tissues (1). Immunocytochemistry using isotype-specific antibodies showed that all members of cPKC are enriched in the brain but their distributions are distinctly different (7). Abundant expression of PKC $\gamma$  in the hippocampal pyramidal cells and cerebellar Purkinje cells (5) has implicated it in the modulation of synaptic plasticity, including long term potentiation (LTP) (21) and long term depression (LTD) (22).

Developmentally, the expression of PKC $\gamma$  is low at birth and increases progressively up to 2–3 weeks (23, 24). In contrast, a considerable amount of PKC $\alpha$  and PKC $\beta$  are expressed before birth in brain, suggesting that PKC $\gamma$  is important for synaptic formation rather than for early neuronal development and that the deletion of PKC $\gamma$  is unlikely to alter early development.

Under electron microscopy, PKC $\gamma$  is localized in the cytoplasm of the soma including nucleus and dendrites including dendritic spines, axon and synaptic terminals (25, 26). This intracellular localization also differs from those of other cPKCs (7), suggesting that the association of PKC isotypes with their specific substrates in different intracellular compartment results in isotype specific function. However, live imaging studies using GFP (green fluorescent protein)-tagged PKC $\gamma$  revealed a rapid cycling of this isozyme between the cytoplasm and plasma membrane in cells upon stimulation of G-protein coupled receptors or Ca<sup>2+</sup>-ionophore (27, 28). The dynamic movement of PKC $\gamma$  in response to various stimuli indicates that PKC $\gamma$  does not always exist in the cytoplasm but interacts with specific substrates in other subcellular compartments when activated. Furthermore, as PKC isotypes show isotype-specific translocation and distinct translocation depending on stimulus (29, 30), the various PKC translocation, in addition to the existence of multiple isotypes, is a basic molecular mechanism of multiple function of this enzyme.

## 3. PKC $\gamma$ specific functions in nervous system

**3-1. LTP.** Among various neuronal functions which involve PKC activation, modulation of synaptic plasticity by PKC has attracted the attention of biochemists and neuroscientists alike. Many papers have implicated the involvement of PKC in LTP, a synaptic model of memory. Activation of PKC by phorbol esters potentiates a synaptic transmission which resembles LTP in hippocampal slices (31–

33). Additionally, direct injection of PKC into the postsynaptic hippocampal pyramidal cells mimics LTP (34). Finally, PKC inhibitors prevents the induction of LTP (35). Thus, the activation of postsynaptic PKC appears to be necessary for the induction of LTP. As PKC $\gamma$  is predominantly localized in the postsynaptic dendrites in the hippocampal pyramidal cells, the data are consistent with involvement of PKC $\gamma$  in LTP.

The generation of PKC $\gamma$  knockout mice was reported in 1993 by Abeliovich *et al.* (8). The PKC $\gamma$  deficient mice are viable and their brain anatomy is normal when examined by light microscopy. Behaviors such as grooming, feeding and mating are also unimpaired, although the mutant mice moved with a mild ataxic gait. The authors first examined the effect of deletion of PKC $\gamma$  on the expression of LTP in the CA1 region of the hippocampus (8). Although synaptic transmission evoked by stimulating hippocampal axons in PKC $\gamma$  deficient mice are indistinguishable from the wild-type mice, in PKC $\gamma$  deficient mice, LTP is rarely induced by the commonly used high frequency stimulation. However, after a low frequency stimulation is used to produce LTD, LTP can be elicited in the knockout mice, suggesting that PKC $\gamma$  regulates LTP but is not necessary for the actual process of synaptic plasticity. In fact, the knockout mice can learn to carry out hippocampus-dependent tasks, although they exhibit mild deficits in spatial and contextual learning (9).

**3-2. LTD.** LTD, the use-dependent decrease in synaptic strength, is the opposite phenomenon of LTP (22, 36). The involvement of PKC $\gamma$  in LTD induction is strongly suggested by reports which show that (i) LTD is blocked by PKC inhibitors (37), (ii) PKC activators such as phorbol ester induced depression of synaptic transmission (37), and (iii) PKC $\gamma$  is the major PKC isotype in Purkinje cells (5). Thus, the deletion of PKC $\gamma$  would be predicted to abolish LTD. However, LTD is fully inducible in the cerebellar slices of the mutant mice (38). It is noteworthy that a PKC inhibitor peptide (PKC19-36) completely blocks LTD in wild-type mice but does not abolish LTD in mutant mice (37, 39). This suggests that PKC $\gamma$  plays a pivotal role in LTD in wild-type mice and that unknown kinases compensate for the PKC $\gamma$  deficiency in the mutant mice. This compensation for the lack in PKC $\gamma$  by other kinase(s) makes it difficult to elucidate the specific function of PKC $\gamma$  in the mutant mice.

PKC $\gamma$  deficient mice exhibit impaired motor coordination but are fully capable of discrete motor learning (40). In mature mutant mice, 40% of Purkinje cells are innervated by multiple climbing fibers. In wild-type mice, these multiple innervations are eliminated during the 3rd week after birth, resulting in one-to-one innervation between the Purkinje cells and climbing fibers. There are several reports of other knockout mice which exhibit this persistent multiple innervation of Purkinje cells by climbing fibers. Mice deficient in metabotropic receptor 1 (mGluR1) (41) or phospholipase C $\beta$ 4 (PLC $\beta$ 4) (42) show a similar phenotype of multiple innervation and motor discoordination. Taken together, the results from these mutant mice suggest that an mGluR1-PLC $\beta$ -PKC $\gamma$  signaling pathway in cerebellar Purkinje cells is involved in the elimination of climbing fibers and the observed motor discoordination is due to the persistent multiple innervation of Purkinje cells by climbing fibers.



**3-3. Modulation of receptor function.** PKC $\gamma$  is also abundant in the dorsal horn of the spinal cord and has been suggested to be important in sensory signal processing including pain. Several studies have shown that activation of  $\mu$ -opioid receptors in the spinal cord induce prolonged PKC translocation (43) and that inhibition of PKC prevents the development of antinociceptive tolerance to  $\mu$ -opioid agonists (44). Of the 10 PKC isotypes, the following evidence for the involvement of PKC $\gamma$  in signaling for pain has been reported: selective  $\mu$ -opioid receptor agonists (i) increase the amount of membrane-associated PKC $\gamma$  but not other PKC isotypes and (ii) desensitize  $\mu$ -opioid receptor-mediated G-protein activation (45, 46).

PKC $\gamma$  deficient mice have been used to demonstrate that activation of PKC $\gamma$  is critical for the development of morphine induced reinforcing (46) and enhancement of nociceptive responses (47). In PKC $\gamma$  deficient mice,  $\mu$ -opioid receptor-mediated analgesia/antinociception is enhanced and functional  $\mu$ -opioid receptors are protected from degradation by phosphorylation (48). Furthermore, PKC $\gamma$  deficient mice failed to develop a neuropathic pain syndrome after partial nerve section (49). These findings suggest that PKC $\gamma$  may contribute to the induction of the psychological dependence on morphine and the development of PKC $\gamma$  specific inhibitor may enable us to alleviate pain by protecting the tolerance. It is also interesting that epinephrine-induced hyperalgesia is also attenuated in mice lacking PKC $\epsilon$ , a presynaptic localized isotype of nPKC (50). Although both PKC $\gamma$  and PKC $\epsilon$  act at different levels of the neuraxis, both isotypes play a role in pain responses.

The modulation of GABA $_A$  receptors by PKC was also reported but the role of PKC in their function is controversial. The response of GABA $_A$  receptors expressed in *Xenopus* oocytes is inhibited by phorbol esters (51) and the mutation of a possible PKC phosphorylation site, Ser343, in the  $\gamma$ 2L subunit of GABA $_A$  receptor reduced the effect of PKC activation (52). In contrast, the activity of GABA $_A$  receptors expressed in fibroblasts are enhanced by active PKC (53).

Ethanol and benzodiazepines are known to enhance the function of GABA $_A$  receptor. The mutation of Ser343 in the  $\gamma$ 2L subunit prevented ethanol potentiation but not benzodiazepine potentiation (54). GABA $_A$  receptors isolated from brain membranes of PKC $\gamma$  deficient mice, do not respond to ethanol, although the deletion of the PKC $\gamma$  gene does not alter their response to muscimol, flunitrazepam or pentobarbital (55). Behaviorally, the mutant mice also display reduced sensitivity to the acute effects of ethanol on righting reflex and body temperature, but show normal responses to flunitrazepam or pentobarbital. Mutant mice consume more ethanol and display decreased tolerance development to the sedative hypnotic and hypothermic effects of ethanol (56, 57), suggesting that these PKC $\gamma$  null mice may be a suitable model for the study of alcoholism. Interestingly, PKC $\epsilon$  also regulates the sensitivity of GABA $_A$  receptor to ethanol. In contrast to PKC $\gamma$  mutant mice, PKC $\epsilon$  deficient mice show supersensitivity to allosteric activation by ethanol and flunitrazepam and exhibit reduced ethanol self-administration (58). These findings suggest that two PKC isotypes, PKC $\gamma$  and PKC $\epsilon$ , modulate the sensitivity of GABA $_A$  receptors to ethanol but have opposite actions on GABA $_A$  receptor activity.

#### 4. Mutation of PKC $\gamma$ gene related to disease

The genomic mapping in patients was performed to search for functional PKC polymorphisms or mutations associated with familial genetic abnormalities. Linkage analysis revealed that a major locus of retinitis pigmentosa (RP) exists at chromosome position 19q, which includes the PKC $\gamma$  gene. Additionally, a point mutation in PKC $\gamma$  that segregates with RP was found in two RP families (59). The mutation is a C-A transversion, which substitutes a serine for an arginine residue at codon 659 in the catalytic domain of PKC $\gamma$ . Although the physiological function of this isotype in retina is unknown, some immunocytochemical studies demonstrate the expression of PKC $\gamma$  in the amacrine and ganglion cells but not in photoreceptor cells (60). The effect of Arg659 mutation on the kinase activity of PKC $\gamma$  is unclear, but it is possible that this mutation affects PKC $\gamma$  maturation, as Arg659 is present between two important residues for maturation, turn motif (Thr655) and hydrophobic motif (Thr674) (61, 62).

Another report suggests that PKC $\gamma$  is a candidate gene for Parkinsonian syndrome. The AS/AGU rat is a spontaneously occurring mutation which exhibits altered behavior and brain pathology resembling Parkinsonian syndrome. At an early age of AS/AGU rat, the extracellular dopamine levels are markedly decreased in the AS/AGU rat. Later, loss of dopaminergic cells in the substantia nigra and dysfunction of movement are evident (63–65). Positional cloning of the agu mutation showed that this locus is tightly linked to the PKC $\gamma$  gene. A comparison of PKC $\gamma$  cDNA sequence from AS/AGU rats and that from the parental AS strain revealed that the G to T exchange at nucleotide 841 alters a GAG (281Glu) codon to an inframe TAG stop codon (66). This new stop codon would truncate the PKC $\gamma$  at 280 amino acids, resulting in expression of most of regulatory domain of PKC $\gamma$ . The generation of knock-in mice expressing this truncated PKC $\gamma$  instead of full length PKC $\gamma$  would help the elucidation of the etiology of neurodegenerative diseases such as Parkinsonian syndrome. It is also noteworthy that LTP in the CA1 region of the hippocampus from AS/AGU rats is not altered (67), while LTP in PKC $\gamma$  deficient mice is impaired under the conventional condition described above. Thus, it is possible that the regulatory domain of PKC $\gamma$  in AS/AGU rats plays a role in LTP.

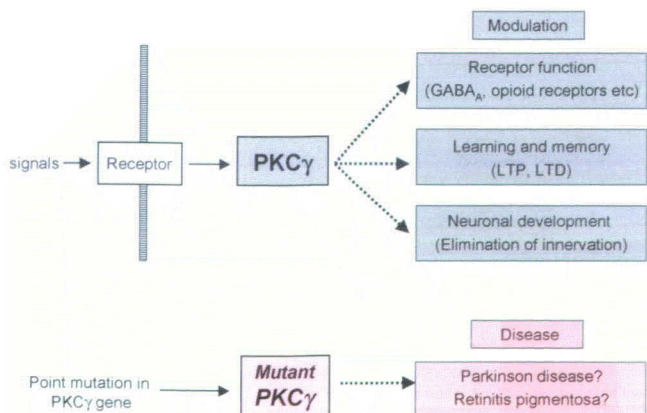


Fig. 1. Possible functions of PKC $\gamma$  in nervous system.

## Conclusion

Recent studies using PKC $\gamma$  mutant mice have accumulated evidences for PKC $\gamma$  specific functions in the nervous system (Fig. 1). However, as described above, PKC $\gamma$  function could be compensated by other kinases, and neuronal network is subtly abnormal in the mutant mice. Further studies utilizing inducible knockouts of PKC $\gamma$  are necessary to fully elucidate the neuronal functions of PKC $\gamma$ .

## REFERENCES

- Nishizuka, Y. (1988) The molecular heterogeneity of protein kinase C and implications for cellular regulation. *Nature* **334**, 661–665
- Nishizuka, Y. (1995) Protein kinase C and lipid signaling for sustained cellular response. *FASEB J.* **9**, 484–496
- Ohno, S. (1997) The distinct biological potential of PKC isotypes in *Protein Kinase C*, pp. 179–188, Springer-Verlag, Heidelberg
- Kikkawa, U., Ono, Y., Ogita, K., Fujii, T., Asaoka, Y., Sekiguchi, K., Kosaka, Y., Igarashi, K., and Nishizuka, Y. (1987) Identification of the structures of multiple subspecies of protein kinase C expressed in rat brain. *FEBS Lett.* **217**, 227–231
- Saito, N., Kikkawa, U., Nishizuka, Y., and Tanaka, C. (1988) Distribution of protein kinase C-like immunoreactive neurons in rat brain. *J. Neurosci.* **8**, 369–382
- Huang, F.L., Yoshida, Y., Nakabayashi, H., Young, I.W.S., and Huang, K.-P. (1988) Immunocytochemical localization of protein kinase C isozymes in rat brain. *J. Neurosci.* **8**, 4734–4744
- Tanaka, C. and Saito, N. (1992) Localization of subspecies of protein kinase C in the mammalian central nervous system. *Neurochem. Int.* **21**, 499–512
- Abeliovich, A., Chen, C., Goda, Y., Silva, A.J., Stevens, C.F., and Tonegawa, S. (1993) Modified hippocampal long-term potentiation in PKC $\gamma$ -mutant mouse. *Cell* **75**, 1253–1262
- Abeliovich, A., Paylor, R., Chen, C., Kim, J.J., Wehner, J.M., and Tonegawa, S. (1993) PKC $\gamma$  mutant mice exhibit mild deficits in spinal and contextual learning. *Cell* **75**, 1263–1271
- Parker, P.J., Coussens, L., Totty, N., Rhee, L., Young, S.E., Chen, S., Stabel, M., Waterfield, D., and Ullrich, A. (1986) The complete primary structure of protein kinase C - the major phorbol ester receptor. *Science* **233**, 853–859
- Coussens, L., Parker, P.J., Rhee, L., Yang-Feng, T.L., Chen, E., Waterfield, M.D., Francke, U., and Ullrich, A. (1986) Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signalling pathways. *Science* **233**, 859–866
- Kofler, K., Erdel, M., Utermann, G., and Baier, G. (2002) Molecular genetics and structural genomics of the human protein kinase C gene module. *Genome Biol.* **3**, 14.1–14.10
- Takanaga, H., Mukai, H., Shimakawa, M., Konishi, H., Kikkawa, U., Koizumi, T., and Ono, Y. (1995) Functional characterization of the promoter region of the mouse protein kinase C $\gamma$  gene. *FEBS Lett.* **368**, 276–278
- Nishizuka, Y. (1986) Studies and perspectives of protein kinase C. *Science* **233**, 305–312
- Cho, W. (2001) Membrane targeting by C1 and C2 domains. *J. Biol. Chem.* **276**, 32407–32410
- Murakami, K. and Routtenberg, A. (1985) Direct stimulation of purified protein kinase C by unsaturated fatty acids (oleate, arachidonate) in the absence of phospholipids and calcium. *FEBS Lett.* **192**, 189–193
- Nishizuka, Y. (1995) Protein kinase C and lipid signaling for sustained cellular responses. *FASEB J.* **9**, 484–496
- Ono, Y., Fujii, T., Igarashi, K., Kuno, T., Tanaka, C., Kikkawa, U., and Nishizuka, Y. (1989) Phorbol ester binding to protein kinase C requires a cysteine-rich zinc-finger-like sequence. *Proc. Natl. Acad. Sci. USA* **86**, 4868–4871
- Irie, K., Nakahara, A., Nakagawa, Y., Ohigashi, H., Shindo, M., Fukuda, H., Konishi, H., Kikkawa, U., Kashiwagi, K., and Saito, N. (2002) Establishment of a binding assay for protein kinase C isozymes using synthetic C1 peptides and development of new medicinal leads with protein kinase C isozyme and C1 domain selectivity. *Pharmacol. Ther.* **93**, 271–281
- Kohout, S.C., Corbalan-Garcia, S., Torrecillas, A., Gomez-Hernandez, J.C., and Falke, J.J. (2002) C2 domains of protein kinase C isoforms  $\alpha$ ,  $\beta$ , and  $\gamma$ : activation parameters and calcium stoichiometries of the membrane-bound state. *Biochemistry* **41**, 11411–11424
- Bliss, T.V.P. and Collingridge, G.L. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**, 31–39
- Ito, M. (1989) Long term depression. *Annu. Rev. Neurosci.* **12**, 85–102
- Hashimoto, T., Ase, K., Sawamura, S., Kikkawa, U., Saito, N., Tanaka, C., and Nishizuka, Y. (1988) Postnatal development of a brain specific subspecies of protein kinase C in rat. *J. Neurosci.* **8**, 1678–1683
- Yoshida, Y., Huang, F.L., Nakabayashi, H., and Huang, K.-P. (1988) Tissue distribution and developmental expression of protein kinase C isozymes. *J. Biol. Chem.* **263**, 9868–9873
- Kose, A., Saito, N., Ito, H., Kikkawa, U., Nishizuka, Y., and Tanaka, C. (1988) Electron microscopic localization of type I protein kinase C in rat Purkinje cells. *J. Neurosci.* **8**, 4262–4268
- Kose, A., Ito, A., Saito, N., and Tanaka, C. (1990) Electron microscopic localization of  $\gamma$ - and  $\beta$ II-subspecies of protein kinase C in rat hippocampus. *Brain Res.* **518**, 209–217
- Sakai, N., Sasaki, K., Ikegaki, N., Shirai, Y., and Saito, N. (1997) Direct visualization of translocation of  $\gamma$ -subspecies of protein kinase C in living cells using fusion proteins with green fluorescent protein. *J. Cell Biol.* **139**, 1465–1476
- Oancea, E. and Meyer, T. (1998) Protein kinase C as a molecular machine for decoding calcium and diacylglycerol signals. *Cell* **95**, 307–318
- Shirai, Y., Kashiwagi, K., Yagi, K., Sakai, N., and Saito, N. (1998) Distinct effects of fatty acids on translocation of gamma- and epsilon-subspecies of protein kinase C. *J. Cell Biol.* **143**, 511–521
- Ohmori, S., Shirai, Y., Sakai, N., Fujii, M., Konishi, H., Kikkawa, U., and Saito, N. (1998) Three distinct mechanisms for translocation and activation of the  $\delta$  subspecies of protein kinase C. *Mol. Cell. Biol.* **18**, 5263–5271
- Linden, D.J., Murakami, K., and Routtenberg, A. (1986) A newly discovered protein kinase C activator (oleic acid) enhances long term potentiation in the intact hippocampus. *Brain Res.* **379**, 358–363
- Linden, D.J., Sheu, F.-S., Murakami, K., and Routtenberg, A. (1987) Enhancement of long-term potentiation by cis-unsaturated fatty acid: relation to protein kinase C and phospholipase A2. *J. Neurosci.* **7**, 3783–3792
- Malenka, R.C., Madison, D.V., and Nicoll, R.A. (1986) Potentiation of synaptic transmission in the hippocampus by phorbol esters. *Nature* **321**, 175–177
- Hu, G.-Y., Hvalby, O., Walaas, S.I., Albert, K.A., Skjeflo, P., Andersen, P., and Greengard, P. (1987) Protein kinase C injection into hippocampal pyramidal cells elicits features of long term potentiation. *Nature* **328**, 426–429
- Malinow, R., Schulman, H., and Tsien, R.W. (1989) Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. *Science* **245**, 862–866
- Linden, D.J. (1994) Long-term synaptic depression in the mammalian brain. *Neuron* **12**, 457–472
- Linden, D.J. and Connor, J.A. (1991) Participation of postsynaptic PKC in cerebellar long-term depression in culture. *Science* **254**, 1656–1659
- Chen, C., Kano, M., Abeliovich, A., Paylor, R., Chen, L., Bao, S., Kim, J.J., Hashimoto, K., Thompson, R.F., and Tonegawa, S. (1995) Impaired motor coordination correlates with persistent multiple climbing fiber innervation in PKC $\gamma$  mutant mice. *Cell* **83**, 1233–1242
- Hemart, N., Daniel, H., Jaillard, D., and Crepel, F. (1995) Receptors and second messengers involved in long term depression in rat cerebellar slices *in vitro*: a reappraisal. *Eur. J. Neurosci.* **7**, 45–53



40. Kano, M., Hashimoto, K., Chen, C., Abeliovich, A., Aiba, A., Kurihara, H., Watanabe, M., Inoue, Y., and Tonegawa, S. (1995) Impaired synapse elimination during cerebellar development in PKC $\gamma$  mutant mice. *Cell* **83**, 1223–1231
41. Kano, M., Hashimoto, K., Kurihara, H., Watanabe, M., Inoue, Y., Aiba, A., and Tonegawa, S. (1997) Persistent multiple climbing fiber innervation of cerebellar Purkinje cells in mice lacking mGluR1. *Neuron* **18**, 71–79
42. Kano, M., Hashimoto, K., Watanabe, M., Kurihara, H., Offermanns, S., Jiang, H., Wu, Y., Jun, K., Shin, H.-S., Inoue, Y., Simon, M.I., and Wu, D. (1998) Phospholipase C $\beta$ 4 is specifically involved in climbing fiber synapse elimination in the developing cerebellum. *Proc. Natl. Acad. Sci. USA* **95**, 15724–15729
43. Narita, M., Makimura, M., Feng, Y.Z., Hoskins, B., and Ho, I.K. (1994) Influence of chronic morphine treatment on protein kinase C activity: comparison with butorphanol and implication for opioid tolerance. *Brain Res* **650**, 175–179
44. Narita, M., Narita, M., Mizoguchi, H., and Tseng, L.F. (1995) Inhibition of protein kinase C, but not of protein kinase A, blocks the development of acute antinociceptive tolerance to an intrathecally administered  $\mu$ -opioid receptor agonist in the mouse. *Eur. J. Pharm.* **280**, R1–3
45. Narita, M., Mizoguchi, H., Narita, M., Nagase, H., Suzuki, T., and Tseng, L.F. (2001) Involvement of spinal protein kinase C $\gamma$  in the attenuation of opioid m-receptor-mediated G-protein activation after chronic intrathecal administration of [D-Ala<sup>2</sup>, N-MePhe<sup>4</sup>, Gly-Ol<sup>5</sup>] enkephalin. *J. Neurosci.* **21**, 3715–3720
46. Narita, M., Aoki, T., Ozaki, Y., Yajima, Y., and Suzuki, T. (2001) Involvement of protein kinase C $\gamma$  isoform in morphine-induced reinforcing effects. *Neuroscience* **103**, 309–314
47. Martin, W.J., Liu, H., Wang, H., Malmberg, A.B., and Basbaum, A.I. (1999) Inflammation-induced upregulation of protein kinase C $\gamma$  immunoreactivity in rat spinal cord correlates with enhanced nociceptive processing. *Neuroscience* **88**, 1267–1274
48. Narita, M., Mizoguchi, H., Suzuki, T., Narita, M., Dun, N.J., Imai, S., Yajima, Y., Nagase, H., Suzuki, T., and Tseng, L.F. (2001) Enhanced  $\mu$ -opioid responses in the spinal cord of mice lacking protein kinase C $\gamma$  isoform. *J. Biol. Chem.* **276**, 15409–15414
49. Malmberg, A.B., Chen, C., Tonegawa, S., and Basbaum, A.I. (1997) Preserved acute pain and reduced neuropathic pain in mice lacking PKC $\gamma$ . *Science* **278**, 279–283
50. Khasar, S.G., Lin, Y.-H., Martin, A., Dadgar, J., MaMahon, T., Wang, D., Hundle, B., Aley, K., Isenberg, W., MacCarter, G., Green, P.G., Hodge, C.W., Levine, J.D., and Messing, P.G. (1999) A novel nociceptor signaling pathway revealed in protein kinase C $\epsilon$  mutant mice. *Neuron* **24**, 253–260
51. Sigel, E. and Baur, R. (1988) Activation of protein kinase C differentially modulates neuronal Na<sup>+</sup>, Ca<sup>2+</sup>, and  $\gamma$ -aminobutyrate type A channels. *Proc. Natl. Acad. Sci. USA* **85**, 6192–6196
52. Krishek, B.J., Xie, X., Blackstone, C., Hagan, R.L., Moss, S.J., Smart, T.G. (1994) Regulation of GABA<sub>A</sub> receptor function by protein kinase C phosphorylation. *Neuron* **12**, 1081–1095
53. Lin, Y.-F., Browning, M.D., Dudek, E.M., and Macdonald, R.L. (1994) Protein kinase C enhances recombinant bovine  $\alpha$ 1 $\beta$ 1 $\gamma$ 2L GABA<sub>A</sub> receptor whole-cell currents expressed in L929 fibroblasts. *Neuron* **13**, 1421–1431
54. Wafford, K.A. and Whiting, P.J. (1992) Ethanol potentiation of GABA<sub>A</sub> receptors requires phosphorylation of the alternatively spliced variant of the  $\gamma$ 2 subunit. *FEBS Lett.* **313**, 113–117
55. Harris, R.A., McQuilkin, S.J., Paylor, R., Abeliovich, A., Tonegawa, S., and Wehner, J.M. (1995) Mutant mice lacking the  $\gamma$  isoform of protein kinase C show decreased behavioral actions of ethanol and altered function of  $\gamma$ -aminobutyrate type A receptors. *Proc. Natl. Acad. Sci. USA* **92**, 3658–3662
56. Bowers, B.J., Owen, E.H., Collins, A.C., Abeliovich, A., Tonegawa, S., and Wehner, J.M. (1999) Decreased ethanol sensitivity and tolerance development in  $\gamma$ -protein kinase C null mutant mice is dependent on genetic background. *Alcohol Clin. Exp. Res.* **23**, 387–397
57. Bowers, B.J. and Wehner, J.M. (2001) Ethanol consumption and behavioral impulsivity are increased in protein kinase C $\gamma$  null mutant mice. *J. Neurosci.* **21**, RC180
58. Hodge, C.W., Mehmert, K.K., Kelley, S.P., MaMahon, T., Haywood, A., Olive, M.F., Wang, D., Sanchez-Perez, A.M., and Messing, R.O. (1999) Supersensitivity to allosteric GABA<sub>A</sub> receptor modulators and alcohol in mice lacking PKC $\epsilon$ . *Nat. Neurosci.* **2**, 997–1002
59. Al-Magthteh, M., Vithana, E.N., Inglehearn, C.F., Moore, T., Bird, A.C., and Bhattacharya, S.S. (1998) Segregation of a PRKCG mutation in two RP11 families. *Am. J. Hum. Genet.* **62**, 1248–1252
60. Osborne, N.N., Barnett, N.D., Moriis, N.J., and Huang, F.L. (1992) The occurrence of three isoenzymes of protein kinase C ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) in retinas of different species. *Brain Res* **570**, 161–166
61. Newton, A.C. (1997) Regulation of protein kinase C. *Curr. Opin. Cell Biol.* **9**, 161–167
62. Shirai, Y. and Saito, N. (2002) Activation mechanisms of PKC: maturation, catalytic activation and targeting. *J. Biochem.* **132**, 663–668
63. Campbell, J.M., Payne, A.P., Gilmore, D.P., Russel, D., McGadey, J., Clarke, D.J., Branton, R., Davies, R.W., and Sutcliffe, R.G. (1997) Age change in dopamine levels in the corpus striatum of Albino Swiss (AS) and AS/AGU mutant rats. *Neurosci. Lett.* **239**, 54–56
64. Campbell, J.M., Gilmore, D.P., Russel, D., Grownay, C.A., Favor, G., Kennedy, A.K., Davies, R.W., Payne, A.P., and Stone, T.W. (2000) Pharmacological analysis of extracellular dopamine and metabolites in the striatum of conscious AS/AGU rats, mutants with locomotor disorder. *Neuroscience* **100**, 45–52
65. Payne, A.P., Campbell, J.M., Russel, D., Favor, G., Sutcliffe, R.G., Bennet, N.K., Davies, R.W., and Stone, T.W. (2000) The AS/AGU rat: a spontaneous model of disruption and degeneration in the nigrostriatal dopaminergic system. *J. Anat.* **196**, 629–633
66. Craig, N.J., Alonso, M.B.D., Hawker, K.L., Shiels, P., Glencorse, T.A., Campbell, J.M., Bennet, N.K., Canham, M., Donald, D., Gardiner, M., Gilmore, D.P., MacDonald, R.J., Maitland, K., MacCallion, A.S., Russel, D., Payne, A.P., Sutcliffe, R.G., and Davies, R.W. (2001) A candidate gene for human neurodegenerative disorders: a rat PKC $\gamma$  mutation causes a Parkinsonian syndrome. *Nat. Neurosci.* **4**, 1061–1062
67. Shahraki, A. and Stone, T.W. (2002) Long-term potentiation and adenosine sensitivity are unchanged in the AS/AGU protein kinase C-deficient rat. *Neurosci. Lett.* **327**, 165–168