MINIREVIEW

GTP-Binding Proteins and Their Regulatory Actions on Ion Channels

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The science of 'signal transduction mechanisms' is the most dramatically progressing field in cell biology during the past several years. Guanine nucleotide-binding proteins (G-proteins) have been identified in almost all cells in every tissue, and their major roles are known to transduce various intracellular signals by interacting between receptor molecules and effector enzymes or ion channels. They regulate synaptic transmission, contractions of cardiac and smooth muscles, secretions of endocrine and exocrine cells, translation system of the messenger RNA, and mitotic cell cycles [17, 21, 58, 61].

This paper describes basic structures and functions of G-proteins and focuses on their modulatory or regulatory actions for ionic channels in the plasma membrane while attempting to put forward some possible unifying concept from the diverse information that has accumulated.

1. BASIC STRUCTURES AND FUNCTIONS OF G-PROTEINS

G-proteins are solubilized from membranes by detergent as monomers of about 100 kDa. Each monomer is composed of α -, β -, and γ -subunits. The β - and γ -subunits are about 35 kDa and 8 kDa, respectively, and not very different among various species of G-proteins [19, 39]. They are so tightly associated to each other that they are inseparable unless they are denatured. The α -subunits are relatively larger molecules of 35–55 kDa, and different among the species of G-proteins. All α -subunits are readily dissociable from $\beta\gamma$ -subunits when G-proteins are activated simply by raising the temperature in GTP-containing solution [11] or by stimulating the receptors to which they are coupled [43, 67]. In the inactive form of G-proteins, GDP is bound to the catalytic site of GTPase localized in α -subunit. The conformational change in the receptor molecule elicited by agonist binding influences the coupling G-protein to release the bound GDP, then intracellular GTP takes the place of the same binding site, as an 'exchange reaction,' and starts to be hydrolyzed to GDP. The kinetics of this hydrolysis is relatively slow. During the hydrolysis, α -

subunit of the G-protein dissociates from $\beta\gamma$ -subunits. This stage is called 'G-protein activation.' This activation is 'switched off' when hydrolyzed GDP reoccupies the catalytic site, and α -subunit reassociates with $\beta\gamma$ -subunits to form stabler conformation of the G-protein. Non-hydrolyzable GTP-analogues such as GTP- γ S and Gpp(NH)p, therefore, can 'switch on' the G-protein activation irreversibly without causing 'switch off.'

Bacterial toxins such as cholera toxin (CTX) and pertussis toxin (PTX) include a protomer subunit which has an adenosine-diphosphate (ADP)-ribose transferase. These toxins can ADP-ribosylate α -subunits of particular G-proteins in the presence of nicotinamide adenine dinucleotide (NAD) and sometimes ADP-ribosylating factor (ARF). For example, CTX ADP-ribosylates α -subunits (α_s) of G_s (G-protein which stimulates adenylate cyclase) at the arginine residue near the GTPase, and activates G_s irreversibly [62]. On the other hand, PTX ADP-ribosylates α -subunits (α_i and α_o) of G_i (G-protein which inhibits adenylate cyclase), and G_o (other G-protein which does not interfere with adenylate cyclase [49]). Generally, G_i is 10 times more abundant than G_s in the plasma membranes of most cells [20], and G_o is 5–10 times more abundant than G_i in the whole brain [18]. The ADP-ribosylation by PTX occurs at the cysteine residue of these α -subunits, and uncouples these G-proteins from their receptors [25, 63, 65]. CTX reduces GTPase activity at the α -subunit of G_s [9], but PTX does not impair GTPase activity at the α -subunit of G_i or G_o [29]. Because of these specific effects of each toxin, they are extremely useful in identifying G-proteins in membranes and in studying G-protein functions.

In the visual system, rhodopsin is a receptor molecule for the light. Photo-activated rhodopsin can activate a G-protein (G_t) called 'transducin.' G_t is sensitive to both CTX and PTX [1, 65]. The activated α_t can stimulate cyclic GMP-phosphodiesterase to reduce intracellular cGMP and hyperpolarize the photo-receptor cells [60]. The $\beta\gamma$ -subunits are generally hydrophobic and localized within a lipid layer of the membrane to anchor the hydrophilic α -subunit, and important for maintaining the contact with each receptor molecule. Regulatory roles of α - and $\beta\gamma$ -subunits for the ion channels are described below.

II. G-PROTEIN-REGULATED OPENING OF K^+ -CHANNELS

(A) α - and $\beta\gamma$ -subunits of G_i or G_o

Applying a single-channel recording technique to the chicken embryonic atrial muscle cells, LOGOTHETIS *et al.* [38] and NEER and CLAPHAM [43] demonstrated that 10^{-9} M order of $\beta\gamma$ -subunits purified from bovine brain G-proteins (G_i or G_o) could open K^+ -channels coupled to the muscarinic receptor of M_2 -type. On the other hand, YATANI *et al.* [72] and other researchers in Brown and Birnbaumer's group [70] showed that 10^{-12} M order of α -subunit purified from human erythrocyte G-proteins (G_i) could open K^+ -channels coupled to the muscarinic receptor of M_2 -type in the guinea pig atrial cell. There were heated debates between these two groups with respect to α - or $\beta\gamma$ -subunits.

Soon thereafter, however, LOGOTHETIS *et al.* [37] concurred that both α - and $\beta\gamma$ -subunits of G_i but not G_i isolated from bovine brain could open K^+ -channels coupled to M_2 -receptors in the chicken embryonic atrial muscles, although they later considered that the action of $\beta\gamma$ -subunits was different from that of α -subunit, as described below.

(B) *Generality of G_i or G_o involvement*

Apart from the problem of α - or $\beta\gamma$ -subunits, the involvement of PTX-sensitive G-protein in K^+ -channel opening was found not to be specific to the channel coupled to the muscarinic receptor (M_2) in the mammalian heart. This was demonstrated by SASAKI and SATO [54, 55] in the ganglion cells of *Aplysia*. They proved that K^+ -channel openings elicited by stimulations of acetylcholine (ACh) (M_2), dopamine (D_2), histamine (H_2), and phenylalanine-methionine-arginine-phenylalanine-amide (FMRFamide) (F_k) receptors are all regulated by PTX-sensitive G-proteins. Similar observations were made in *Aplysia* by other investigators [6, 66]. In mammals, K^+ -channel openings were elicited by stimulations of serotonin (5HT-1A)- and GABA (B)-receptors in the hippocampal pyramidal cells of the rat [3], and by stimulations of adenosine receptors in atrial myocytes [33], and somatostatin receptors in pituitary tumor cells [69] or locus coeruleus neurons of the rat [26]; K^+ -channel openings were elicited by stimulations of noradrenaline (α_2) and opioid (μ and δ) receptors in the locus coeruleus neurons [2] and neuroblastoma-glioma hybrid cells [52]. All were found to be regulated by PTX-sensitive G-proteins.

Historically, G_i , which inhibits adenylate cyclase, was the first G-protein found to be sensitive to PTX. Later, also G_o and some G-proteins which are considered to stimulate phospholipase C or A_2 were found to be sensitive to PTX [8, 44]. Activations of these G-proteins should trigger a variety of biochemical changes inside the cell, such as a decrease in cAMP, increase or decrease in Ca^{2+} , inositol trisphosphate (IP_3), diacylglycerol (DAG), arachidonic acid (AA), and their metabolites. However, it should be remembered that the K^+ -channel openings triggered by activation of G_i or G_o could occur independently from intracellular biochemical changes mentioned above, although the latter changes could have some modulatory effects on the former.

(C) *Definitions of G_k and G_p and their functions*

Some G-proteins which are responsible for stimulation of phospholipase C and resultant phosphatidylinositol (PI) turnover, are also sensitive to PTX. For example, the stimulation of bradykinin receptors in NG108-15 cells activates this kind of G-protein and induces the opening of Ca^{2+} -dependent K^+ -channels, but this is subsequently blocked by activation of protein kinase C [7]. However it should be noted that the opening of Ca^{2+} -dependent K^+ -channels is not the direct result of G-protein activation. Instead, it is simply due to an increase in intracellular Ca^{2+} released from the endoplasmic reticulum after activation of phospholipase C.

Accordingly, this G-protein should be different from other G-proteins described above.

It should also be remembered that the openings of K^+ -channels coupled to many receptors are all sensitive to PTX, but PTX-sensitive G-proteins are not always directly capable of opening the K^+ -channels. Therefore, it is reasonable to have the name ' G_k ' for the G-protein that is sensitive to PTX and directly capable of opening K^+ -channels (not Ca^{2+} -dependent K^+ -channels), as suggested by Brown and Birnbaumer's group [70,72], although it creates a little confusion with the term G_k which is used to mean a potassium conductance in neurophysiology. The α -subunit synthesized with cDNA for G_k was found to be identical to α_i -3, although α_i -1 and α_i -2 were both capable of opening K^+ -channels [72].

Stimulations of the adrenergic receptor of α_1 -type, muscarinic receptor of M_1 -type, thyrotropin-releasing hormone (TRH) receptor of GH_3 cells, and thrombin receptor of fibroblast cells are known to activate phospholipase C. They are sensitive to neither CTX nor PTX, and never open K^+ -channels (Ca^{2+} -independent) during their responses [36]. Generally the G-proteins which activate phospholipase C but are not sensitive to either CTX or PTX seem to be incapable of directly opening the K^+ -channel (Ca^{2+} -independent), although the reason for this is not well understood at this moment; this group of G-proteins are considered to be different from G_k , and called ' G_p .' Accordingly, the name G_p should be used distinctly from other G-proteins which stimulate phospholipase C but are sensitive to either CTX or PTX (See IV. below). G_k seems to be different from other G_i or G_o because G_k activation scarcely causes the stimulation of PI turnover in the cells so far examined. Is the G_k never capable of metabolizing any phospholipid?

Recently, there is accumulating evidence suggesting that G_k might stimulate phospholipase A_2 . Agonist-induced stimulations of the adrenergic receptor of α_2 -type, opiate receptor of δ -type, and muscarinic receptor of M_2 -type are known to activate G_k , as mentioned above. Interestingly, these stimulations also cause a slight increase (0.3) in intracellular pH in all cells examined. This increase in pH has been considered to be the result of G_k -induced acceleration of Na^+/H^+ exchange. These changes would cause an increase in intracellular Ca^{2+} and alkalization, both being favorable for activation of phospholipase A_2 [4, 35]. For example, stimulation of FMRFamide receptors of *Aplysia* sensory neuron activates G_k to produce K^+ -dependent hyperpolarization. During this response, many lipoxygenase products such as labeled 12-HPETE and 5-HETE were secreted from the cell and detected by high-performance liquid chromatography (HPLC) [5, 47]. It was even considered that these lipoxygenase metabolites were primarily responsible for activating the K^+ -channels because the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA) effectively blocked the FMRFamide-induced hyperpolarization. As mentioned earlier, Neer and Clapham's group of investigators initially described that $\beta\gamma$ -subunits of G_k are responsible for opening the K^+ -channels coupled to the muscarinic receptors of atrial muscles [38]. More recently, they described that $\beta\gamma$ -subunits of G_k activate phospholipase A_2 to release arachidonic acid (AA) from the

lipid membrane [30]. AA is further metabolized by lipoxygenase to produce 5-HPETE, 12-HETE, and leukotrienes (B_4 , C_4) [32]. They also think that these lipoxygenase products are primarily responsible for opening the K^+ -channels of atrial myocytes [30]. The $\beta\gamma$ -subunits of G_i are also known to activate phospholipase A_2 in the rod outer segments [27], though their ability to open K^+ -channels remains unknown.

(D) Physiological and pharmacological properties of the G_k -dependent K^+ -channels

G_k -dependent K^+ -channels recorded from atrial muscles were first considered to have an inward rectifying characteristic. However, more precise analysis with a single-channel recording proved that the kinetics of K^+ -channel opening by ACh was considerably different from that of inward rectifying K^+ -channel [53], and does not show much inward rectification within the voltage range from -100 to -25 mV. On the other hand, KURACHI *et al.* [32] described that the K^+ -channel opened by AA [38] exhibited a strong inward rectification in the same cardiac myocyte. This is rather an interesting discrepancy, because K^+ -channels coupled to ACh, histamine, FMRFamide, and dopamine in *Aplysia* neurons demonstrated practically no signs of inward rectification (our unpublished observation). Definitely, they are not the Ca^{2+} -activated K^+ -channels. They are not readily blocked by tetraethylammonium (TEA) or apamin, but depressed by aminopyridines or simply by lowering the temperature [22]. They are similar to S- or M-channels because intracellular injections of cAMP or cGMP significantly depress the opening of these K^+ -channels. Phorbol esters also depress these K^+ -channel activities [10]. These findings suggest that protein kinases A, G, and C can phosphorylate either these K^+ -channels or G_k to deactivate them. In fact, G_k has been proved to be a good substrate for protein kinase C [28].

III. G-PROTEIN-REGULATED OPENING OF Na^+ - AND Ca^{2+} -CHANNELS

(A) Voltage-dependent nature of these ion channels

A number of receptors for neurotransmitters and hormones have been known to stimulate the adenylate cyclase and cause excitation or secretion in many neurons and secretory cells. These excitatory and secretory effects are generally associated with the occurrence of so-called 'slow inward current' across these receptor membranes. The ionic mechanisms underlying this 'slow inward current' have been ascribed to the opening of voltage-dependent Na^+ -channels or Ca^{2+} -channels, and/or the concomitant closing of the specific K^+ -channels (S-channels). The exact identification of ion channels involved in this slow inward current is very difficult because these Na^+ - and Ca^{2+} -channels are voltage dependent and often exhibit a characteristic negative conductance when activated by receptor stimulation [40]. When these responses are examined under the whole cell voltage-clamp at potentials near the resting level, the apparent slope conductance of the membrane often

decreases during the slow inward current [40], as if K^+ -channels were closed. Even with the patch-clamp technique, the openings of these Na^+ - and Ca^{2+} -channels and concomitant closing of K^+ -channels, if it occurs, would be difficult to demonstrate them all at the same time.

(B) *Roles of cAMP and cGMP*

Intracellular injection of cAMP or cGMP into the cells of olfactory cilia produces slow inward current and this response is considered as the direct action of cyclic nucleotide to open voltage-dependent Na^+ -channels, rather than mediated by cyclic nucleotide-dependent protein kinase A or G [51]. The cyclic GMP-induced depolarizing responses of the cone and rod in retina are also considered as the direct action of cGMP to open voltage-dependent Na^+ -channels and not due to the activation of protein kinase G [51]. Not only these primary sensory cells, but many neurons in the *Aplysia* ganglion respond to both cyclic AMP and GMP with a prompt activation of voltage-dependent Na^+ -channels but not Ca^{2+} -channels even after the treatment with inhibitors for protein kinases A and G (our unpublished observation). Further studies are needed to explain these direct effects of cyclic nucleotides, which are independent of protein kinase activations.

(C) *Roles of protein kinases A and G*

In cardiac myocytes, intracellular injection of the catalytic subunit of protein kinase A increases an open probability of the voltage-dependent Ca^{2+} -channels [45]. In fact, the dihydropyridine-sensitive Ca^{2+} -channel complex purified from skeletal muscles has been proved to be actually phosphorylated by protein kinase A [16]. In snail neurons, intracellular injection of the protein kinase G increases the activity of voltage-dependent Ca^{2+} -channels as well as the 5HT-induced Ca^{2+} -current [46]. Accordingly, it can be said that both cyclic nucleotides themselves and their related protein kinases (A and G) act synergistically to open voltage-dependent Na^+ - and Ca^{2+} -channels when receptors are stimulated. In addition, it should be remembered that the K^+ -channel activities regulated by G_k could be depressed at the same time by activated protein kinase A or G as mentioned above.

(D) *Direct action of α_s subunit*

Further surprising experiments were performed by investigators in Brown and Birnbaumer's group after they completed their work on G_k . They demonstrated that the α -subunit of G_s purified from the human erythrocyte membranes can activate the dihydropyridine-sensitive Ca^{2+} -channels of the cardiac myocyte as well as the Ca^{2+} -channels of skeletal muscle T-tubules incorporated into the planar lipid bilayers [41, 71]. The most important fact they discovered is that the purified α_s of nm order can activate these Ca^{2+} -channels rather directly, being independent of actions of cyclic AMP and protein kinase A. The experiments of G_s synthesis with cDNA in *Escherichia coli* revealed at least four different types of α -subunits: S-1, S-2, L-1, and L-2 (S and L denote the shorter and longer peptide chains, respectively)

[41]. They proved that not only S-1 type but also S-2 and L-1 types of α -subunits could activate Ca^{2+} -channels, although they were less potent than natural α_s . They concluded that the activation of G_s by receptor stimulation induces stimulatory effects on both the adenylate cyclase and voltage-dependent Ca^{2+} -channels at the same time, each effector being activated independently but synergistically for the ultimate activation of Ca^{2+} -channels.

IV. ACTIVATIONS OF PHOSPHOLIPASE C AND THEIR EFFECTS ON ION CHANNELS

There are at least three different subtypes in the family of G-proteins which stimulate phospholipase C. First of all, G_p is a substrate for neither PTX nor CTX. The receptors which are likely to be coupling with G_p are described above. PTX-sensitive G-proteins which stimulate phospholipase C are coupled with the formylpeptide receptor in the myeloid differentiated HL-60 cells, α_1 -adrenergic receptor in adipocytes, thrombin receptor in hamster fibroblast cells, and bradykinin receptor in dorsal ganglion hybrid cells and neuroblastoma $\times$ glioma 108 cells. CTX-sensitive G-proteins which stimulate phospholipase C are coupled with the antigen receptor in T-cell line Jurkat, secretin receptor in rat pancreatic acinar cells, and vasopressin receptor in WRK-1 mammary tumor cells [36]. The effect of CTX on the adenylate cyclase system is a persistent activation of the cyclase, whereas CTX does not activate phospholipase C but inhibits receptor-stimulated PI turnover.

Phospholipase C is the enzyme which hydrolyzes phosphatidylinositol biphosphate to produce IP_3 and DAG. IP_3 can be further phosphorylated by intrinsic enzymes to form IP_4 , IP_5 , and IP_6 . IP_3 is known to act on the endoplasmic reticulum and release Ca^{2+} from the storage site. The increase in intracellular Ca^{2+} concentration should cause the openings of Ca^{2+} -activated K^+ -channels or Ca^{2+} -activated Cl^- -channels. These were actually proved in the cultured neuroblastoma $\times$ glioma hybrid cells when phospholipase C was activated by bradykinin receptor stimulation [7], and in *Xenopus* oocyte when phospholipase C was activated by 5HT and ACh [12]. DAG is known to activate protein kinase C, which has been reported to block the activities of Ca^{2+} -dependent K^+ -channels as well as G_k -operated K^+ -channels [14]. In fact, protein kinase C can phosphorylate PTX-sensitive G-proteins and deactivate them directly, thus blocking all G_i -, G_o -, and G_k -dependent reactions [28]. These effects of protein kinase C are always seen in all responses to stimulations of a variety of receptors which are considered to stimulate PI turnover, regardless of whether their G-proteins are sensitive to PTX or not. Peculiarly, however, the opening of Ca^{2+} -activated K^+ -channels is seen only when PTX-sensitive G-proteins (G_i or G_o) are activated as in the cases of neuroblastoma $\times$ glioma hybrid cells and *Xenopus* oocytes, but never observed in the responses elicited by activation of G_p . Whether this is due to the difference of G-proteins or to the modulatory effects of other effector enzymes remains to be solved.

V. G-PROTEIN-REGULATED CLOSING OF Ca^{2+} -CHANNELS(A) *Activations of PTX-sensitive G-proteins*

The activities of voltage-dependent Ca^{2+} -channels are generally depressed whenever G_k is activated by various receptor stimulations or by GTP- γ S applied intracellularly. This was confirmed by opiate receptor of neuroblastoma glioma cells [23], α_2 -receptor [50], dopamine-receptor [64], GABA-B-type receptor of the dorsal root ganglion cell [13], and somatostatin receptor of pituitary cell line [34]. These depressing effects were all inhibited by PTX and GDP- β S. The Ca^{2+} -channels that were depressed by these receptor stimulations were T-type and N-type channels while the L-type channels were not depressed significantly. It is of interest that the α -subunits of G_o and G_i also have the same depressing effects on these Ca^{2+} -channels, though the potency of G_o is 10 times greater than that of G_i [23]. These effects of G_k and G_o are considered to be the direct effects on the Ca^{2+} -channels, and not due to the changes in activities of the adenylate cyclase system or of the protein kinase system.

HORNE *et al.* [24] extracted a crude Ca^{2+} -channel complex from the triads of skeletal muscle of the rabbit, and isolated the Ca^{2+} -channel activity component from its regulatory component. They proved that the G_o -dependent block occurred in the membrane reconstituted with the Ca^{2+} -channel activity component isolated from its regulatory component. SCOTT and DOLPHIN [56] demonstrated in the dorsal root ganglion cells that intracellular injection of GTP- γ S depressed Ca^{2+} -channel activities of the T- and N-types but not the L-type. Surprisingly, Ca^{2+} -antagonists such as D600, nifedipine, and diltiazem markedly augmented Ca^{2+} -current in the presence of GTP- γ S [56]. They interpreted this as the result of Ca^{2+} -antagonists binding to the agonist binding sites during the activation of G_o by GTP- γ S.

Quite recently, I had a chance to meet with members of the USSR Academy of Sciences in Moscow. According to Prof. Fesenko, the director of the Institute of Biological Physics, Pushchino, 1–3 μM α -subunit of transducin (G_t) may directly block the cGMP-mediated opening of cationic (Na^+ - and Ca^{2+} -) channels in the outer segment membrane of the rod [31]. This effect was confirmed with the excised membrane patch in the presence of Br-cGMP and GTP- γ S.

(B) *Involvement of protein kinase C?*

Direct suppressing effects of PTX-sensitive G-proteins on the opening of cationic channels were described above. However, there is a certain evidence that the activation of protein kinase C, as secondary to the activations of these G-proteins, is responsible for the suppressing effects on the cationic channels.

Using the dorsal root ganglion cells dissected from 11-day chicken embryos, RANE and DUNLAP [48] demonstrated that DAG analogue 1,2-oleoylacetyl glycerol (OAG) and phorbol ester 12-deoxyphorbol 13-isobutyrate (DPB) effectively depressed the voltage-gated Ca^{2+} -current. Incubation of dorsal root ganglion cells with OAG prevented further reduction in Ca^{2+} -current by norepinephrine, which is

known to inhibit the voltage-dependent Ca^{2+} -channel in these cells. From these results they suggested that the transmitter-induced inhibitions of Ca^{2+} -current observed in dorsal root ganglion cells might be due to the activation of protein kinase C.

The neuropeptide Y (NPY) is found in high concentrations in the dorsal horn of the spinal cord, and known to be the most potent inhibitor of the voltage-gated Ca^{2+} -current in the dorsal root ganglion cells [15]. NPY stimulates the synthesis of DAG by dorsal ganglion cells and activates protein kinase C. This peptide can depress not only the N- or T-type Ca^{2+} -current but also the L-type Ca^{2+} -current. However, it depressed mainly N- or T-type Ca^{2+} -current when examined after protein kinase C was deactivated by down regulation [15].

VI. NEUROPHYSIOLOGICAL RELEVANCE

Apart from the involvement of protein kinase C, the fact that activation of G_o inhibits the activity of N-type Ca^{2+} -channel is very important for the new concept of presynaptic inhibition, because N-type Ca^{2+} -channels are rich in presynaptic terminals and responsible for the control of transmitter release [42]. Presynaptic inhibition can occur by blocking Ca^{2+} -influx through N-type Ca^{2+} -channels when receptors on the terminal membranes are stimulated by any neurotransmitters or hormones to activate G_o . It is no longer important whether presynaptic terminals are depolarized or hyperpolarized by these receptor stimulations.

The most abundant G-protein in the brain is G_o , comprising 1% of total membrane proteins [59]. G_o is particularly rich in the hippocampus and frontal cortex, though it distributes widely in other areas of the brain. It is of interest that the G_o distribution is parallel with the distribution of protein kinase C in the brain [68]. This suggests the important roles of PI responses in the brain functions, causing mobilizations of Ca^{2+} inside the neuron, and phosphorylations of ion channels or their regulatory proteins.

A single neuron in the brain receives thousands of synaptic inputs on the cell body and dendrites. It acts as an integrating unit of all synaptic inputs impinging on it. It also receives hormonal or neurohumoral control which would alter the entire mode of integration. For example, if norepinephrine is liberated from a neighboring varicosity by stimulation of locus coeruleus neuron, it may react with extrajunctional α_1 -receptors which are coupled to G_o and activate protein kinase C in a certain neuron. Then, all the inhibitory input activities which would stimulate G_k to produce hyperpolarization in the neuron could be suppressed because protein kinase C would deactivate G_k and K^+ -channel activities. In contrast, the excitatory input activities which would stimulate G_s to produce depolarization in the same neuron could be augmented if protein kinase C is mildly activated just to phosphorylate and deactivate G_i [57]. However, they could be rather depressed if protein kinase C is activated intensely to phosphorylate the receptor protein itself [58]. Other synaptic inputs which would stimulate phospholipase A_2 to cause

arachidonic cascade reactions could also be suppressed because protein kinase C would deactivate G_k , G_o , and G_i .

A single receptor molecule may be coupled to multiple G-proteins, and a single G-protein may be coupled to multiple effector enzymes and ion channels. These complexed biochemical changes are all integrated to determine the ultimate excitability and firing pattern of the single neuron, which is conveyed to the succeeding neuron as processed information. The basic studies on the regulatory roles of G-proteins for various ion channels are important not only for understanding the signal transduction mechanisms in a single cell level but also for providing the molecular basis for information processing in the brain, such as long-term potentiation or inhibition, habituation, and heterosynaptic facilitation, etc.

Key words: G-proteins, ion channels, receptors, PTX, CTX.

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