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COMMENTARY

Regulation of Ionotropic Receptors by Protein Phosphorylation

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ABSTRACT. The regulation of synaptic signal transduction is of central importance to our understanding of normal and abnormal nervous system function. One mechanism by which signal transduction can be affected is the modification of cellular sensitivity by alterations of transmembrane receptor properties. For G-protein coupled receptors, protein phosphorylation is intimately involved in many stages of receptor regulation. This appears to be true for ionotropic receptors as well. Evidence of a role for protein kinase and protein phosphatase activity in the multi-staged ionotropic receptor regulation cascade is presented and a comparison to G-protein coupled receptor regulation is considered. BIOCHEM PHARMACOL 51;11:1417–1425, 1996.

KEY WORDS. ionotropic receptors; receptor regulation; protein kinase; protein phosphatase; trafficking; sequestration; degradation; synthesis; neuropathology

Intercellular communication is mediated by the release of chemical messenger molecules and the interaction of these messengers with specialized receptor proteins on target cells. Numerous receptors have been characterized and assigned to six receptor superfamilies based on structure and function [1]. Of these, the ionotropic receptor superfamily (a.k.a. ligand-gated ion channels) provides the basis for rapid synaptic transmission in the nervous system. Depending on the nature of the ionotropic receptors activated, the resulting changes in membrane permeability produce ion currents leading to depolarization and neural excitation or hyperpolarization and neural inhibition. Secondary to the changes in membrane potential, ligand–ionotropic receptor interactions also initiate important regulatory events that modulate cellular responsiveness to further stimulation. Some aspect of the signal transduction cascade initiates a series of events that ultimately result in changes in receptor properties. The regulation of receptor properties by ligand–receptor interactions is likely to be a key component in the control of sensitivity of transmembrane signaling processes [2, 3]. The importance of receptor regulation is underscored by Hollenberg: ". . . receptor regulation *per se* must now be considered just as much a part of the responsiveness of a system to an agonist as is an end-organ response like muscle contraction or acid secretion" [2]. For neurons the equivalent output is action potential generation. Although a number of mechanisms for post-translational receptor modification have been described [4], protein phosphorylation is likely the most widespread and, by far, the best studied.

The present review will focus on the regulation of ionotropic receptor properties by phosphorylation and dephosphorylation reactions. The modulation of synaptic transmission by ionotropic receptor phosphorylation may be a key process underlying molecular mechanisms of learning and memory [5–9]. Further, the misregulation of transmembrane signaling may also be an important component in some neuropathological disorders [10, 11].

MULTIPLE STAGES OF RECEPTOR REGULATION

The control of cellular sensitivity to agonists is a multi-staged process and has been best characterized for G-protein coupled receptors. The cellular responsiveness to many neurotransmitters and hormones is diminished rapidly during prolonged exposure to agonists. G-protein coupled receptors initially show a rapid decline (maximal effects within approximately 15 min of exposure) in agonist-stimulated second messenger production (i.e. "desensitization") [12, 13]. Desensitization is followed by internalization of the receptors in the continued presence of agonist (approx. 30 min to several hours for maximal effects) [12, 14, 15]. If agonist stimulation is then halted, the receptors may be recycled back to the cell membrane [14]. Stimulation maintained for long periods (several hours to days) leads to changes in receptor synthesis and degradation rates [12, 15–18]. Receptor phosphorylation by specific protein kinases appears to play a key role in all stages of these regulatory events [3, 13, 15, 19].

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All of the above processes are obviously important in the regulation of cellular responsiveness and are generally referred to as receptor regulation. Agonist-dependent regulation is dependent on activation of receptors as the initial event, and is alternatively termed homospecific or homologous regulation. Additionally, activation of one type of receptors may lead to regulation of another receptor type, a phenomenon variously termed heterospecific or heterologous regulation or transmodulation.

Recent evidence suggests that ionotropic receptors also display three distinct phases of regulation analogous to those described for G-protein coupled receptors. For ionotropic receptors, desensitization is observed as a rapid decline in receptor-mediated current responses [20, 21]. Rapid desensitization of $GABA_A$* receptor-mediated currents has been reported [22–24]. More prolonged agonist exposure (1 hr) promotes sequestration of $GABA_A$ receptors [25], probably by endocytosis via clathrin-coated vesicles [26]. Chronic exposure (2–7 days) of neurons to GABA produces a down-regulation of $GABA_A$ receptors [27, 28] with recovery antagonized by protein synthesis inhibitors [27].

Using the model described above for G-protein coupled receptor regulation, we will attempt to show that ionotropic receptor regulation shares several common features. Key among these is that protein kinases and phosphatases play important roles in regulation.

FUNCTIONAL REGULATION OF IONOTROPIC RECEPTORS BY PROTEIN PHOSPHORYLATION

The macromolecular protein complex that forms an ionotropic receptor may be described generally as having two major functional domains: (i) the ion-channel, and (ii) various ligand binding domains, including the agonist binding site(s) and, in some cases, modulatory binding sites. Such binding sites serve to regulate the function of the associated ion-channel in response to extracellular stimuli (e.g. neurotransmitters and modulators). Several properties of ionotropic receptors may be modified to produce modulation of ionotropic electrophysiological properties. The postsynaptic current (I) in response to activation of a particular population of ion channels is described by Levitan [29] as: $I = i \cdot p \cdot N$, where i is the unitary current characteristic of the ion channel type, p is the probability of channel opening, and N is the number of functional channels in the membrane. In the case of ligand-gated ion channels, p is determined largely by the availability of agonist and the affinity of the receptor for the agonist. Modulation of any or all of these properties will produce changes in the current response (I) at the whole-cell level.

Electrophysiological investigations have demonstrated the influence of protein kinase activity on ionotropic receptor function (see Table 1). The consequences of kinase activation are diverse and include: potentiation or reduction of receptor-mediated currents and changes in the rate of current decay in the continued presence of agonist (i.e. desensitization kinetics). Additionally, receptor phosphorylation appears to be necessary for the maintenance of functional integrity for some receptors. In a phenomenon termed "rundown," receptor-mediated current responses gradually decline, even in the absence of agonist. This rundown effect can be prevented by inclusion of factors to maintain receptor phosphorylation (e.g. ATP, Mg^{2+}, phosphatase inhibitors) in the recording pipette [44–48], suggesting that it results from the loss of diffusible factors necessary for the maintenance of protein kinase activity.

The functional consequences of phosphorylation of a particular receptor type appear to depend on the specific kinases involved (see Table 1), the subunit composition of the receptors [23, 24], and the editing of RNA transcripts encoding receptor subunit proteins [23, 24, 38]. The potentiation or reduction of ionotropic receptor-mediated currents may be accounted for by changes in single channel properties such as channel open probability [30, 37], open frequency [33, 41], mean open time [41], and changes in desensitization rates [23, 24, 49, 50].

The changes in functional characteristics of ionotropic receptors in response to protein kinase activity coincide, in many cases, with the direct phosphorylation of specific receptor subunit proteins [23, 24, 31, 34, 36, 43, 49, 50], although the involvement of receptor-associated regulatory phosphoproteins has not been ruled out in all cases. In cases where the major phosphorylation sites on receptor subunit proteins have been identified, the second cytoplasmic loop region (which connects the second and third transmembrane domains) appears to be a common target [5, 7, 21]. However, this may not be true for the glutamate ionotropic receptors since this domain appears to be extracellular [51]. Regardless of the specific sites of phosphorylation, the majority of ionotropic receptors are functionally regulated by direct phosphorylation of receptor subunit proteins.

HOMOLOGOUS AND HETEROLOGOUS REGULATION OF IONOTROPIC RECEPTORS

The consequences of receptor phosphorylation are not limited to regulation of receptor-mediated currents. Radioligand binding studies have revealed that several other aspects of ionotropic receptor regulation appear to be under the control of receptor phosphorylation and dephosphorylation.

* *Abbreviations:* GABA, γ-aminobutyric acid; PKA, cAMP-dependent protein kinase; PKC, Ca^{2+}-phospholipid-dependent protein kinase; PKT, protein tyrosine kinase; Cam KII, Ca^{2+}-calmodulin-dependent protein kinase type II; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; NMDA, *N*-methyl-D-aspartate; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; SR95531, 2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl) pyridazinium bromide; PKA-IP, cyclic AMP-dependent protein kinase inhibiting peptide; Sp-cAMPs, Sp-cyclic 3′,5′-hydrogen phosphorothioate adenosine triethylammonium; Rp-cAMPs, Rp-cyclic 3′,5′-hydrogen phosphorothioate adenosine triethylammonium; NaBD, sodium β-glycerophosphate; NaV, sodium orthovanadate; AP, alkaline phosphatase; KN-62, 1-[*N*,*O*-bis(1,5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine; and GRK, G-protein coupled receptor kinase.

Using a cortical slice binding assay [52], we examined the homologous (agonist-dependent) and heterologous regulation (by non-receptor-mediated depolarization) of the excitatory AMPA-preferring subtype of glutamate receptors (using [^{3}H]CNQX) and inhibitory $GABA_A$ receptors (using [^{3}H]SR95531). For both of these competitive antagonists, labeling is restricted to cell surface receptors, and the changes in binding reflect changes in this receptor pool. Exposure of adult rat cortical slices to the agonist muscimol resulted in a significant decrease in $GABA_A$ receptor number [53, 54]. Incubation in medium containing the agonists AMPA or quisqualate produced a significant decrease in AMPA receptor number [54, 55] (Fig. 1). For AMPA receptors, response desensitization appeared to occur in concert with decreases in specific [^{3}H]CNQX binding.*

Sustained cellular depolarization induced by veratridine has profound effects on the level of radioligand binding of many receptor populations [56, 57]. Veratridine is a sodium (Na^+) channel toxin that blocks channel inactivation leading to sustained cellular depolarization [58]. AMPA receptors respond to pharmacological depolarization by veratridine with a decrease in functional receptor number in adults [54, 55]. In contrast, the inhibitory $GABA_A$ receptors showed increases in number in adult cortex [53, 54] (Fig. 1). We have proposed elsewhere that the reciprocal responses of excitatory AMPA and inhibitory $GABA_A$ receptor populations in the mature nervous system represent a form of neuronal homeostasis, such that the overall cellular response to sustained depolarization acts to dampen the responsiveness of the cell to further depolarization [59].

These experiments demonstrated the homologous and heterologous regulation of cell surface ionotropic receptors. We next sought to determine if protein kinase or phosphatase activity are involved in such processes.

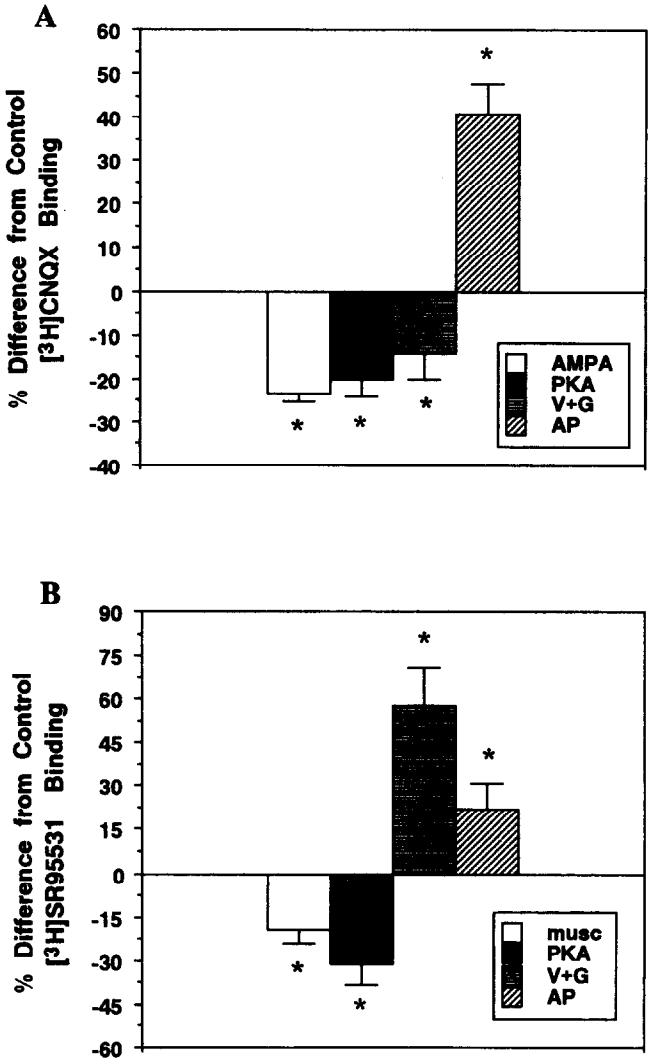

FIG. 1. Effects of agonists, neural activity, and kinase/phosphatase activity on ionotropic receptors in adult rat neocortex slices. (A) Slices were exposed to AMPA (10 μM), the constitutively active catalytic subunit of cAMP-dependent protein kinase (PKA, 1 × 10^{-8} mg/mL), veratridine with glutamate (V + G, 10 μM each) or alkaline phosphatase (AP, 2.5 × 10^{-7} mg/mL) for 120 min at 37°, followed by two 30-min rinses. Slices were incubated with a 10 nM concentration of the AMPA receptor antagonist [^{3}H]CNQX for 180 minutes at 4°. (B) Slices were exposed to the $GABA_A$ agonist muscimol (musc., 10 μM), PKA, V + G, or AP as above. Following treatment and rinse, slices were incubated in 5 nM [^{3}H]SR95531 for 90 min at 4°. Data are normalized to percentage where control represents 100%. Values are expressed as percent difference from specific binding in control (untreated) slices. Values represent the averaged data ± SEM from 6–12 experiments. Each experiment represents the mean of triplicate measures of specific binding. Asterisks indicate a significant difference from control ($P < 0.05$, unpaired t-test). For further details, see Refs. 52–55 and 60.

REGULATION OF CELL SURFACE IONOTROPIC RECEPTORS BY KINASE AND PHOSPHATASE ACTIVITY

Exposure of freeze/thaw permeabilized slices to the catalytic subunit of PKA produced concentration-dependent decreases in $GABA_A$ and AMPA receptor number (Fig. 1), which are blocked by the addition of a specific PKA inhibiting peptide (PKA-IP) [60]. The results of these experiments were further confirmed by experiments using intact cortical slices employing a specific, membrane-permeable activator of PKA. When endogenous stores of PKA are activated in intact cortical slices by Sp-cAMPS, AMPA receptor binding is reduced and this regulation can be blocked by the PKA inhibitor Rp-cAMPs [61]. Activation of Cam KII (but not PKC) also reduces AMPA receptor binding.

Further evidence for the involvement of protein phosphorylation reactions in the modification of ionotropic receptors was provided by experiments using a catalytically active AP to dephosphorylate receptor proteins. Exposure of permeabilized cortical slices to AP produced the opposite effect to that seen for the kinase treatments described

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above. Both $GABA_A$ and AMPA receptor number were increased in a concentration-dependent fashion by AP treatment [54, 60] (Fig. 1). For both receptor populations, this increase could be blocked completely by inhibitors of protein phosphatase activity.

The time courses and magnitudes for regulation of AMPA and $GABA_A$ receptors by kinase/phosphatase activity were very similar to those observed for agonist or veratridine regulation. In all cases, maximal effects were observed within 2 hr and maximal effects fell within 50–150% of control binding. To determine if regulation by agonists or veratridine was mediated by kinase/phosphatase activity, we next attempted to block homologous and heterologous regulation with kinase and phosphatase inhibitors.

KINASE DEPENDENCE OF HOMOLOGOUS REGULATION AND PHOSPHATASE DEPENDENCE OF HETEROLOGOUS REGULATION

The homologous regulation of $GABA_A$ receptors could be blocked by coincubation with PKA-IP. The increase in $GABA_A$ receptors induced by veratridine could be blocked by the phosphatase inhibitors NaBD or NaV. For AMPA receptors, where both agonist and depolarizing stimuli produce decreased AMPA receptor number, the effects of both stimuli can also be blocked by PKA-IP [54].

One concern in such studies is the permeability of the pharmacological agents employed. This is particularly true where the use of intact cortical slices is required in order to observe agonist or veratridine-induced receptor regulation (Fig. 2). For intact slices it is unclear how a peptide inhibitor such as PKA-IP could cross the plasma membrane to inhibit intracellular PKA activity. To clarify this potential confounding variable, experiments were repeated using membrane permeable protein kinase inhibitors. In agreement with our previously published data [61], Rp-cAMPs blocked the regulation of AMPA receptors in response to agonist or veratridine stimulation. The membrane permeable Cam KII specific inhibitor KN-62 also prevented regulation of AMPA receptors. The permeable PKC inhibitor bisindolylmaleimide was ineffective.

Overall, these data provide strong evidence that the activities of endogenous protein kinases (and phosphatases) are involved in the activity-dependent regulation of ionotropic receptors. The effects of kinase and phosphatase activity were observed as changes in the numbers of cell surface receptors. In all cases, maximal regulation was observed within 30–120 min, corresponding to the time frame involved in receptor internalization and recycling events. In the majority of reports summarized in Table 1, the maximal effects of protein kinase activity on ionotropic receptor-mediated currents develop with a similar time course (20–60 min) [23, 24, 31, 32, 35, 36]. In agreement with the model of Sibley *et al.* [3], our results indicate that protein kinase activity promotes decreases in cell surface receptor

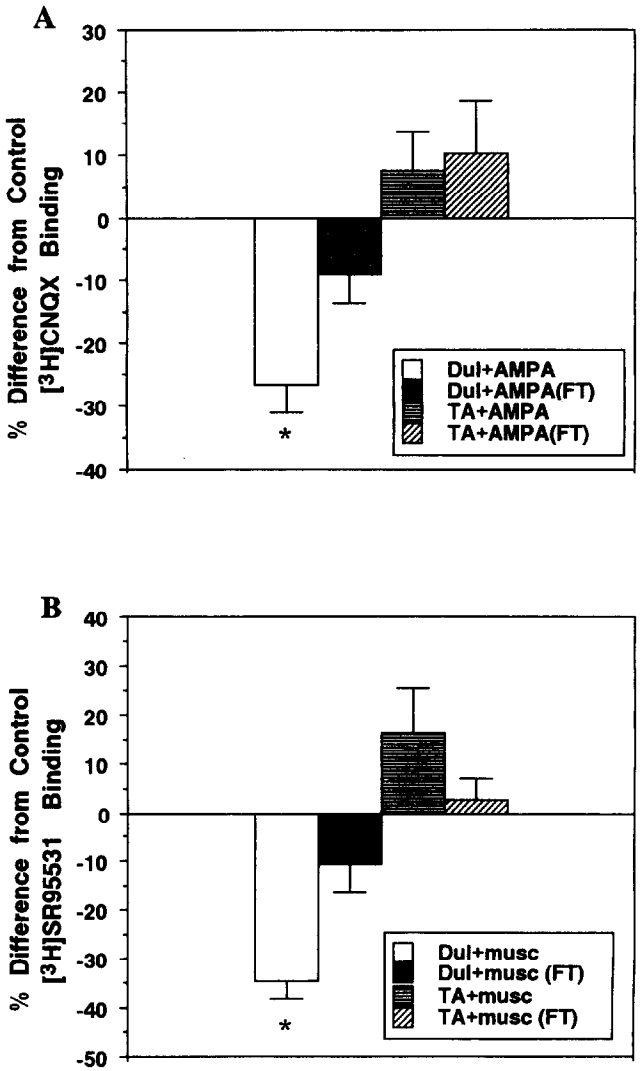

FIG. 2. Effect of cellular integrity and medium composition on ionotropic receptor regulation by agonists in adult rat neocortical slices. (A) Agonist-dependent regulation of AMPA receptors was assayed in intact slices in modified Dulbecco's PBS [52] (Dul + AMPA) or in Tris acetate buffer (TA + AMPA; 150 mM, pH 7.4) to determine if ion removal affects regulation. AMPA-induced regulation was also assayed in freeze–thaw permeabilized slices (FT) to determine if cellular integrity is necessary for regulation. Details of radioligand labeling were as described in the legend of Fig. 1A. Values represent the means ± SEM from 6–8 experiments. (B) $GABA_A$ receptors were treated with muscimol as the agonist in the conditions described in panel A. Values represent the means ± SEM from 4 experiments. Radioligand binding was performed as described in the legend of Fig. 1B. For both panels A and B, asterisks indicate a significant difference from control ($P < 0.05$, unpaired *t*-test). Only intact cells in a physiological buffer display agonist-dependent regulation. Data for both panels are normalized to percentage where control represents 100%. Values are expressed as percent difference from specific binding in control (untreated) slices.

number, whereas dephosphorylation has the opposite effect. Whether the regulation we have observed involves sequestration and recycling of receptors has not been shown directly. However, data from other studies of ionotropic re-

TABLE 1. Modulation of Ionotropic Receptor-Mediated Currents in Response to Protein Kinase Activity

Receptor	Kinase	Consequences	Ref.
Glycine	PKA	Potentiation	30, 31
	PKC	Reduction	31, 32
$GABA_A$	PKA	Reduction	23, 33, 34
		Potentiation	35
	PKC	Reduction	24
Kainate	PKA	Potentiation	36
NMDA	PKC	Potentiation	37, 38
	PKT	Potentiation	39
AMPA	PKA	Potentiation	40–42
	CaM KII	Potentiation	43

Potentiation and reduction refer to the peak amplitude of whole cell currents. This table is not meant to represent a comprehensive review.

ceptor regulation under similar conditions suggest a mechanism of this sort [25, 26].

ACTIVATORS OF PHOSPHORYLATION REACTIONS INVOLVING IONOTROPIC RECEPTORS

For G-protein coupled receptors, receptor activation initiates the production of intracellular second messengers, which in turn may activate enzymes involved in the regulation of cell surface receptors [3, 13]. This feedback mechanism may account for some forms of heterologous regulation of ionotropic receptors but is unlikely to be involved in homologous forms of regulation or transmodulation of one ionotropic receptor type by another. How then can extracellular stimuli such as agonists or depolarization produce activation of the intracellular enzyme (e.g. kinase, phosphatase) pools responsible for the subsequent modulation of receptor function?

One common feature of the stimuli employed to induce regulation of cortical ionotropic receptors is their ability to cause membrane potential shifts. Changes in membrane potential reflect ionic currents across the cell membrane following changes in the membrane permeability to specific ions. Thus, the membrane potential shifts induced by agonists or veratridine equate to transient changes in local ionic concentrations within neurons, which may, in turn, activate intracellular enzymes. To prevent depolarization, Dulbecco's PBS buffer was replaced with Tris acetate buffer, and some of the above experiments were repeated. For both $GABA_A$ and AMPA receptors, regulation could not be induced in Tris acetate buffer [61] (Fig. 2). Furthermore, in freeze–thaw permeabilized slices no regulation by agonists or veratridine was observed for either receptor (Fig. 2). These experiments indicate that the ionic currents generated by AMPA, muscimol, or veratridine are necessary for the regulation of AMPA and $GABA_A$ receptors and demonstrate that occupation of receptors alone is not sufficient to induce regulation.

By supplementing Tris acetate incubation buffer with increasing concentrations of either Na^+, K^+, Ca^{2+}-acetate, or choline chloride, we assayed the effects of each of these ions on AMPA and $GABA_A$ receptor binding. Freeze-thaw permeabilized slices were used to ensure equilibration of the intracellular environment with the incubation medium. For $GABA_A$ receptors, Na^+ caused a concentration-dependent increase in binding that could be blocked by phosphatase inhibitors, whereas Cl^- caused a concentration-dependent decrease in binding that was abolished by kinase inhibition [62] (Fig. 3). For AMPA receptors, Cl^- caused a concentration-dependent increase in binding that was blocked by phosphatase inhibition, and Ca^{2+} caused a concentration-dependent suppression of binding that was blocked by kinase inhibitors [63, 64] (Fig. 3). These studies demonstrate that novel forms of receptor control over protein kinases and phosphatases may occur by the very ions that carry current through their own channels.

A GENERAL MODEL OF IONOTROPIC RECEPTOR REGULATION

Based on the data presented in the previous sections, we offer the following model of regulation of brain ionotropic receptors. The model suggests that regulation occurs with these stages: (i) neurotransmitter stimulation leads to local increases in specific intracellular ionic concentrations; (ii) these ions, in turn, activate specific intracellular enzyme pools leading to the covalent modification of ionotropic receptors; (iii) the phosphorylation or dephosphorylation of receptor subunit proteins results in receptor regulation, probably by sequestration or recycling. The net result is an alteration in the ability of the cell to respond to future stimuli affecting that receptor. The entire process is reversible in similar fashion by enzyme activity of the opposite type. Decreases in receptor number are under the control of specific kinases (perhaps analogous to the GRKs involved in G-protein coupled receptor regulation [65]), which are activated by the ionic species that flow through that receptor's ion channels or through secondary activation of voltage-gated ion channels. Conversely, increases in number are governed by phosphatases activated by an opposing receptor's ionic current, e.g. chloride ions from GABAergic stimulation activate a phosphatase that dephosphorylates AMPA receptors, as well as a kinase acting on their own receptor. Similarly, Na^+ current through AMPA-operated channels activates phosphatases controlling $GABA_A$ receptors and activates a Ca^{2+}-dependent kinase (possibly Cam KII) via secondary activation of voltage-gated Ca^{2+} channels [61].

Although the suggestion that ionotropic receptors may each be regulated by unique kinases and phosphatases may seem quite speculative at present, we note that the existence of other receptor kinases, such as the GRK family of kinases [65] and the $GABA_A$ receptor-associated kinase [66], has been established. The notion that ions other than calcium can modulate kinase and phosphatase function may also seem highly speculative. However, recent data

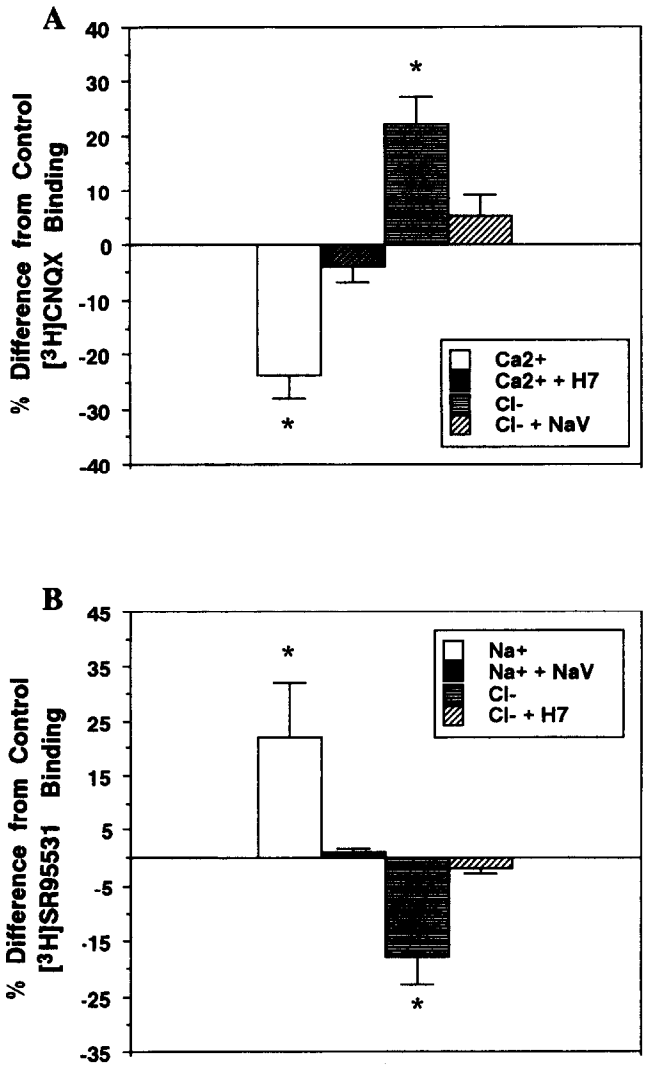

FIG. 3. Ionic determinants of kinase or phosphatase regulation of ionotropic receptors in adult rat neocortical slices. (A) Freeze-thawed slices were exposed to Tris acetate buffer supplemented with calcium acetate or choline chloride at maximally effective concentrations previously shown to affect AMPA receptors [63]. The effect of these ions was determined in the absence or presence of non-specific kinase (H7, 100 μM) or phosphatase (NaV, 1 mM) blockers. See the legend of Fig. 1A for details of radioligand binding. Values represent the means ± SEM of 4–6 determinations. Asterisks represent a significant difference from control ($P < 0.05$, unpaired *t*-test). (B) $GABA_A$ receptors were labeled following exposure to Tris acetate supplemented with sodium acetate or choline chloride [62] in the absence or presence of kinase or phosphatase blockers. Analysis as in panel A, radiolabeling as in Fig. 1B. Data for both panels are normalized to percentage where control represents 100%. Values are expressed as percent difference from specific binding in control (i.e. TA buffer only) slices.

suggest the importance of ions other than calcium in receptor regulatory processes. For example, the depolarization-induced down-regulation of cortical muscarinic acetylcholine receptors occurs via a K^+-dependent mechanism [57]. The agonist-induced down-regulation of nicotinic acetylcholine receptors in cultured rat myotubes is dependent on Na^+ influx [67]. It is also interesting to note that the Na^+ dependency for cerebellar long-term depression [68], a process that requires protein kinase activity and is manifest as a reduction in AMPA receptor function [8]. To us, the notion of specific ionically triggered regulatory enzymes is intriguing, now only supported by indirect evidence, yet warranting further study.

PHARMACOLOGICAL IMPLICATIONS OF RECEPTOR REGULATION FOR NEUROPHYSIOLOGY AND NEUROPATHOLOGY

Phosphorylation events are involved in many aspects of ionotropic receptor regulation. The immediate response of many ionotropic receptors to protein kinase activity is the modulation of desensitization kinetics and, on a longer term, the modulation of cell surface receptor expression. The prolonged exposure of receptors to physiological concentrations of agonist is probably much more relevant to clinical conditions rather than in normal function, e.g. in tachyphylaxis—the progressive decrease in response following repeated administration of therapeutic agents. Perhaps the best known example of this phenomenon comes from the treatment of patients afflicted with encephalitis lethargica, (a.k.a. the "sleeping-sickness"). Sacks has described the remarkable, but transient, improvement in the condition of many such patients upon treatment with levo-dihydroxypenylalanine (L-DOPA) [69]. Although Sacks stresses the complex psychosocial factors influencing the response of individual patients to treatment, biochemical processes such as receptor regulation undoubtedly play an important role as well.

Regulation of specific receptor populations may arise due to the activities of novel kinases and phosphatases that are sensitive to changes in intracellular ions other than calcium. Our results, though indirect, demonstrate that phosphotransferase activities can also be stimulated by Cl^- and Na^+ in a concentration-dependent fashion [62, 63], and the effect of these activities resembles the agonist- or depolarization-induced regulation of AMPA and $GABA_A$ receptors [53–55]. We speculate that such enzymes are involved in the regulation of functional cell surface ionotropic receptor number, thus accounting for the homologous and heterologous regulation of ionotropic receptors by conditions arising following receptor activation. Since different receptor populations are regulated by different ions, members of this putative family of ionotropic receptor kinases and phosphatases may have a requirement for agonist-occupation for catalytic activity to occur (as is the case for GRKs [65]) or may be receptor specific, in terms of their substrate specificity. This does not preclude the importance of multifunctional kinases and phosphatases in ionotropic receptor regulation; clearly, such reactions are important in the heterologous regulation of receptor properties.

Identification and characterization of the elements in receptor regulatory cascades should provide new targets for

pharmacological intervention in neuropathological diseases. This approach may prove particularly useful in the future treatment of conditions such as epilepsy, where the underlying pathology involves the development of hyperexcitability within specific neuronal populations [70]. The anticonvulsant properties of glutamate receptor antagonists have been demonstrated in a variety of animal models of epilepsy [71, 72]. In acute ischemic brain insults such as strokes or following seizure activity, much of the damage observed is thought to result from the over-activation of glutamatergic transmission resulting in excitotoxic cell death [73–76]. Experiments in tissue culture and animal models have demonstrated the neuroprotective effects of glutamate ionotropic receptor antagonists [72, 73, 75]. Although present data are less compelling, glutamate-mediated excitotoxicity may also be involved in several chronic neurodegenerative disorders [74, 75, 77]. The therapeutic benefit of intervention in these conditions may be enhanced by co-administration of agents promoting receptor internalization or degradation along with the antagonists.

An understanding of the basic stages in ionotropic receptor regulation may thus provide a viable framework for future studies of synaptic function and dysfunction. The consideration that receptor regulatory dynamics will be at play during the treatment of patients with drugs designed to enhance or suppress synaptic transmission via particular receptor populations is warranted. Such drugs will undoubtedly alter the activation state of target cells and thus are very likely to promote regulation of the target receptor population (homospecific regulation) as well as other receptor types on the same, and other, cells (heterospecific regulation). Failure to consider the regulatory effects of therapeutic agents may lead to poor responses to treatment, development of unwanted side-effects, or even an exacerbation of the original condition.

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