

Appendix A14.10

Cholinergic System

Acetylcholine (ACh) is the only major low molecular weight neurotransmitter substance that is not derived from an amino acid (Kandel et al., 2000). ACh is synthesized from acetyl CoA and choline in nerve terminals via the enzyme choline acetyl transferase (ChAT). Choline is transported into the brain by uptake from the bloodstream, and enters the neuron via both high-affinity and low-affinity transport processes. (Cooper et al., 2001) In addition to the “standard” ChAT pathway, there are several additional possible mechanisms by which ACh can be synthesized; the precise roles of these additional pathways and their physiological relevance in the CNS remains to be fully elucidated (Cooper et al., 2001). The highest activity of ChAT is observed in the interpeduncular nucleus, caudate nucleus, corneal epithelium, retina, and central spinal roots. In contrast to the other transmitters discussed thus far (which are most dependent on reuptake mechanisms), the ACh signal is terminated primarily by the enzyme acetylcholine esterase, which degrades ACh. Not surprisingly, therapeutic strategies to increase synaptic ACh levels (e.g. for the treatment of Alzheimer’s disease) have focused on inhibiting the activity of cholinesterases.

Several cholinergic pathways have been proposed, but until recently the circuits had not been worked out in the brain because of the lack of appropriate techniques. More recently, the development of tract tracing and histochemical techniques has provided a clearer picture of the cholinergic pathways. In brief, cholinergic neurons can act as local circuit neurons (interneurons) and are found in the caudate-putamen, nucleus accumbens, olfactory tubercle, and Islands of Calleja complex (Cooper et al., 2001). They do, however, also serve to function as projection neurons that connect different brain regions; one fairly well characterized pathway runs from the septum to the hippocampus. The basal forebrain cholinergic complex is composed of cholinergic neurons originating from the medial septal nucleus, diagonal band nuclei, substantia inominata, magnocellular preoptic field, and nucleus basalis. These nuclei project cholinergic neurons to the entire nonstriatal telencephalon, pontomesencephalotegmental cholinergic complex, thalamus, and other diencephalic loci. Descending cholinergic projections from these nuclei also innervate pontine and medullary reticular formations, deep cerebellar and vestibular nuclei, and cranial nerve nuclei (Cooper et al., 2001).

Cholinergic Receptors

There are two major distinct classes of cholinergic receptors, the *muscarinic* and *nicotinic* receptors. Five muscarinic receptors (M1-M5) have been cloned (Kandel et al., 2000). These receptors are G-protein coupled and act either by regulating ion channels (in particular K^+ or

Ca^{2+}) or are linked to second messenger systems. Generally speaking, M_1 , M_3 , and M_5 are coupled to PI hydrolysis whereas M_2 and M_4 are coupled to inhibition of adenylyl cyclase and to regulate K^+ and Ca^{2+} channels (Cooper et al., 2001).

By contrast, the nicotinic receptors are ionotropic receptors, and at least seven different functional receptors (based on different subunit composition) have been identified. Biochemical and biophysical data indicate that the nicotinic receptors in the muscle are formed from five protein subunits, with the stoichiometry of $\alpha_2\beta\gamma\delta$ (Kandel et al., 2000). The binding of ACh molecules on the α subunit is necessary for channel activation. By contrast, neuronal nicotinic receptors contain only two types of subunits (α and β) with the α occurring in at least seven different forms and the β in three (Cooper et al., 2001). Nicotinic receptors may be the targets of considerable cross-talk, as a variety of kinases (including PKA, PKC, and tyrosine kinases) are able to regulate the sensitivity of this receptor. From a clinical standpoint, Freedman et al. (1997) demonstrated that, in a cohort of patients with schizophrenia, abnormal P50 auditory evoked potentials were linked to a susceptibility loci for this disease on chromosome 15. Notably, this is where a nicotinic receptor subunit is found, providing indirect support for the long-standing contention that the high rates of cigarette smoking in schizophrenics may represent (at least in part) an attempt to correct an underlying nicotinic receptor defect.

Glutamatergic System

Glutamate and aspartate are the two major excitatory amino acids in the CNS, and are present in high concentrations (Nestler et al., 2001;). As the principal mediators of excitatory synaptic transmission in the mammalian brain, they participate in wide-ranging aspects of both normal and abnormal CNS function. Physiologically, glutamate appears to play a prominent role in synaptic plasticity, learning, and memory. However, glutamate can also be a potent neuronal excitotoxin under a variety of experimental conditions, triggering either rapid or delayed neuronal death. Unlike the monoamines which require transport of amino acids through the blood brain barrier, glutamate and aspartate cannot adequately penetrate into the brain from the periphery and are produced locally by specialized brain machinery. The metabolic and synthetic enzymes responsible for the formation of these non-essential amino acids are located in glial cells as well as neurons (Nestler et al., 2001). The major metabolic pathway in the production of glutamate is derived from glucose and the transamination of α -ketoglutarate; however, a small proportion of glutamate is formed directly from glutamine. The latter is actually synthesized in glia, via an active process (requiring ATP), and is then transported to neurons where glutaminase is able to convert this precursor to glutamate. Following release, the concentration of glutamate in the extracellular space is highly regulated and controlled, primarily by a sodium-dependent reuptake mechanism involving several transporter proteins. The major glutamate transporter proteins found in the CNS include excitatory amino acid transporters (EAAT): EAAT1 (or GLAST-1), EAAT2

(or GLT-1), and EAAT3 (or EAAC1), with EAAT2 being the most predominantly expressed form in the forebrain. Additionally, these transporters are differentially expressed in specific cell types, with EAAT1 and EAAT2 being found primarily in glial cells, EAAT3 being localized in neurons and EAAT4 is mainly localized in cerebellum. The physiological events regulating the activity of the glutamate transporters are not well understood, although there is evidence that phosphorylation of the transporters by protein kinases may differentially regulate glutamate transporters and therefore glutamate reuptake (Casado et al., 1993; Conradt and Stoffel, 1997; Pisano et al., 1996). Glutamate concentrations have been shown to rise to excitotoxic levels within minutes following traumatic or ischemic injury, and there is evidence that the function of the glutamate transporters becomes impaired under these excitotoxic conditions (Faden et al., 1989). Moreover, microdialysis studies have shown that severe stress increases extracellular levels of glutamate in hippocampus, and N-methyl-D-aspartate (NMDA) glutamate receptor antagonists attenuate stress-induced atrophy of CA₃ pyramidal neurons. Not surprisingly, considerable research has investigated the possible efficacy of drugs which reduce excessive glutamatergic neurotransmission for the treatment of conditions associated with excitotoxicity; to date none have shown clear efficacy in large scale clinical trials.

Glutamatergic Receptors

The many subtypes of glutamatergic receptors in the CNS can be classified into two major subtypes – the ionotropic and metabotropic receptors. The ionotropic glutamate receptor ion channels are assemblies of homo or hetero-oligomeric subunits integrated into the neuron's membrane. Every channel is assembled of (most likely) four subunits associated into a dimer of dimers as has been observed in crystallographic studies (Ayalon and Stern-Bach, 2001; Madden, 2002). Every subunit consists of an extracellular amino-terminal and ligand binding domain, three transmembrane domains and a re-entrant pore loop (located between the first and second transmembrane domains), and an intracellular carboxyl-terminal domain (Hollmann et al., 1994). The subunits associate through interactions between their amino-terminal domains forming a dimer that undergoes a second dimerization mediated by interactions between the ligand binding domains and/or between transmembrane domains (Ayalon and Stern-Bach, 2001; Madden, 2002). Three different subgroups of glutamatergic ion channels have been identified utilizing their pharmacological ability to bind different synthetic ligands, each of which is composed of a different set of subunits. These are the N-methyl-D-Aspartate receptor (NMDA R), α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA R) and kainate receptor (KA R). The latter two groups are often referred to together as the “non-NMDA” receptors, but undoubtedly subserve unique functions. In the adult mammalian brain, NMDA and AMPA glutamatergic receptors are co-localized in approximately 70% of the synapses (Bekkers and Stevens, 1989). By contrast, at early stages of development, synapses are more likely to contain only NMDA

receptors. Radioligand binding studies have shown that NMDA and AMPA receptors are found in high density in the cerebral cortex, hippocampus, striatum, septum, and amygdala.

NMDA Receptors

The NMDA receptor is activated by glutamate and requires the presence of a co-agonist, namely glycine or D-serine, to be activated. However, the binding of both glutamate and glycine is still not sufficient for the NMDA receptor channel to open, since – at resting membrane potential -- the NMDA ion channel is blocked by Mg^{2+} ions. Only when the membrane is depolarized (e.g., by the activation of AMPA or kainate receptors on the same postsynaptic neuron) is the Mg^{2+} blockade relieved. Under these conditions, the NMDA receptor channel will open and permit the entry of both Na^+ and Ca^{2+} .

The NMDA receptor channel is composed of combination of NR1, NR2A, NR2B, NR2C, NR2D, NR3A and NR3B subunits. The binding site for glutamate has been localized to the NR2 subunit and the site for the co-agonist glycine has been localized to the NR1 subunit which is required for receptor function. Two molecules of glutamate and two of glycine are thought to be necessary to activate the ion channel. Within the ion channel, two other sites have been identified called the sigma (σ) site and the phencyclidine (PCP) site. The hallucinogenic drug PCP, ketamine, and the experimental drug dizocilpine (MK-801), all bind at the latter site and are considered noncompetitive receptor antagonists that inhibit NMDA receptor channel function. In preclinical studies, drugs of this type have been shown to have neuroprotective properties against anoxia and hypoglycemia; these studies await clinical confirmation. In clinical psychiatric studies, ketamine has been shown to transiently induce psychotic symptoms in schizophrenic patients, and to produce rapid antidepressant effects in depressed patients (Krystal et al., 2002). These latter observations have led to the investigation of NMDA antagonists as putative novel antidepressants (Krystal et al., 2002; Manji et al., 2003a).

NMDA receptors play a critical role in regulating synaptic plasticity (Malenka and Nicoll, 1999). The best studied forms of synaptic plasticity in the CNS are long-term potentiation (LTP) and long-term depression (LTD) of excitatory synaptic transmission. The molecular mechanisms of LTP and LTD have been extensively characterized and have been proposed to represent cellular models of learning and memory (Malenka and Nicoll, 1999). Induction of LTP and LTD in the CA1 region of the hippocampus and in many regions of the brain has now clearly been demonstrated to be dependent on NMDA-receptor activation. During NMDA-receptor-dependent synaptic plasticity, Ca^{2+} influx through NMDA receptors can activate a wide variety of kinases and/or phosphatases that in turn modulate synaptic strength. An important recent development is the finding that two of the primary molecules involved— Ca^{2+} /calmodulin dependent protein kinase II (CaMKII) and the NMDA subtype of glutamate receptor—form a tight complex with each other at the synapse (Lisman and McIntyre, 2001). Interestingly, this

binding appears to enhance both the autophosphorylation of the kinase and the ability of the entire holoenzyme, which has twelve subunits, to become hyperphosphorylated (Lisman and McIntyre, 2001). This hyperphosphorylated state has been postulated to represent a “memory switch” which can lead to long-term strengthening of the synapse by multiple mechanisms. One important mechanism involves direct phosphorylation of the glutamate-activated AMPA receptors, which increases their conductance. Furthermore, CaMKII, once bound to the NMDA receptor, may organize additional anchoring sites for AMPA receptors at the synapse.

While the NMDA receptor clearly plays important roles in plasticity, abundant evidence has shown that excessive glutamatergic signaling is also involved in neuronal toxicity. With anoxia or hypoglycemia, the highly energy dependent uptake mechanisms that keep glutamate compartmentalized in presynaptic terminals fail. Within minutes, glutamate is massively released into the synaptic space resulting in activation of excitatory amino acid receptors. This leads to depolarization of target neurons via AMPA and kainate receptors and then to inappropriate and excessive activation of NMDA receptors. Considerable data suggests that the large excess of Ca^{2+} entering cells via the NMDA receptor channel may represent an important step in the rapid cell death that occurs via excitotoxicity.

AMPA Receptors

The AMPA receptor is stimulated by the presence of glutamate and characteristically produces a fast excitatory synaptic signal that is responsible for the initial reaction to glutamate in the synapse. In fact, as discussed above, it is generally believed that it is the activation of the AMPA receptor that results in neuronal depolarization sufficient to liberate the Mg^{2+} cation from the NMDA receptor, thereby permitting its activation. The AMPA receptor channel is composed of the combination of GluR1, GluR2, GluR3, and GluR4 subunits, and requires two molecules of glutamate to be activated. AMPA receptors have a lower affinity for glutamate than the NMDA receptor, thereby allowing for more rapid dissociation of glutamate and therefore a rapid deactivation of the AMPA receptor (reviewed in Dingledine et al., 1999).

Recent studies have indicated that AMPA-receptor subunits are direct substrates of protein kinases and phosphatases. Phosphorylation of the receptor subunits regulates not only the intrinsic channel properties of the receptor, but also regulate the interaction of the receptor with associated proteins that modulate the membrane trafficking and synaptic targeting of the receptors (discussed in Malinow and Malenka, 2002). Additionally, protein phosphorylation of other synaptic proteins has been proposed to indirectly modulate AMPA-receptor function by affecting the macromolecular complexes that are important for the presence of AMPA receptors at the synaptic plasma membrane (Malinow and Malenka, 2002; Nestler et al., 2001). Recent studies have been elucidating the cellular mechanisms by which AMPA receptor subunit insertion and trafficking occurs, and have revealed two major mechanisms. (Nestler et al., 2001; Malinow and

Malenka, 2002). The first mechanism is utilized for GluR1 containing AMPA receptor insertion, and is regulated by activity. The second mechanism is governed by constitutive receptor recycling, mainly through GluR2/3 heteromers in response to activity-dependent signals. Recent data is suggesting that AMPA receptor subunit trafficking may play important roles in neuropsychiatric disorders. Thus, Nestler and associates have shown that the ability of drugs of abuse to elevate levels of the GluR1 subunit of AMPA glutamate receptors in the ventral tegmental area (VTA) of the midbrain is crucial for the development of sensitization (Carlezon and Nestler, 2002). They have demonstrated that even transient increases in GluR1 levels within VTA neurons can trigger complex cascades of other molecular adaptations in these neurons and, within larger neural circuits, can cause enduring changes in the responses of the brain to drugs of abuse. Most recently, chronic lithium and valproate have been shown to reduce GluR1 expression in hippocampal synaptosomes, effects which may play a role in their delayed therapeutic effects (Szabo et al., 2002; Du et al., 2004).

Kainate Receptor

The kainate receptor has pre and postsynaptic roles, sharing some properties with AMPA receptors. It is composed by the combination of GluR5, GluR6, GluR7, KA1 and KA2 subunits. The precise role(s) of the KA receptor in the mature CNS remains to be fully elucidated although their activity clearly plays a role in synaptic function in many brain areas.

Metabotropic Glutamate Receptors

The metabotropic glutamate receptors (mGluRs) are G protein coupled receptors. Currently eight types of receptors have been cloned, which can be organized into three different subgroups (Groups I, II, and III) based largely upon the signaling transduction pathways that they activate. These receptors have a large extracellular N-terminal consisting of two lobes forming a “Venus flytrap” binding pocket involved in glutamate recognition and a cysteine rich extracellular domain that connects with seven transmembrane domains separated by short intra and extracellular loops. The intracellular loop plays an important role in the coupling with and selectivity of the G-protein. The cytoplasmic carboxyl-terminal domain is variable in length and is involved with G-protein activation and coupling efficacy (Bruno et al., 2001; Conn and Pin, 1997).

The mGluR Group I includes the mGluR1 (a, b, c, d), and mGluR5 (a, b) receptors preferentially interact with the $G\alpha_{q/11}$ subunit of G-proteins, leading to activation of the inositol trisphosphate/calcium and diacylglycerol/protein kinase C cascades. The receptors are located on both pre- and post-synaptic neurons. Group II metabotropic receptors include mGluR 2 and mGluR 3, which have been best characterized to inhibit adenylyl cyclase, but as with many receptors coupled to G_i/Go , may also regulate ion channels. Group III receptors include mGluR 4

(a, b), mGluR 6, mGluR 7 (a, b), and mGluR 8 (a, b) and are reported to produce inhibition of adenylyl cyclase as well, but also to interact with the phosphodiesterase enzyme regulating guanosine monophosphate (cGMP) levels (Cooper et al., 2001; Squire et al., 2003). The Group II and III receptors are located in the presynaptic membrane and because of their coupling with Gi/o-proteins appear to negatively modulate glutamate and GABA neurotransmission output when activated (i.e. serve as inhibitory auto- and hetero- receptors). Preclinical studies suggest that mGlu II/III receptors are “extrasynaptic” in their localization. That is, that they are located some distance removed from the synaptic cleft, and are thus activated only under conditions of excessive (pathological) glutamate release, when there is sufficient glutamate to diffuse out of the synapse to these receptors (Schoepp, 2001). In preclinical studies, mGluR 2/3 agonists have been demonstrated to exert anxiolytic, antipsychotic and neuroprotective properties (Schoepp, 2001).

Glycine

Glycine is a non-essential amino acid that also functions as a neurotransmitter in the CNS. Although the exact metabolic pathway for glycine production has yet to be fully elucidated, evidence suggests that glycine may be produced in the CNS by two distinct pathways. Firstly, glycine is produced from serine by serine-trans-hydroxy-methylase, in a reversible folate-dependent reaction (Cooper et al., 2001; Nestler et al., 2001). Additionally, glycine may be also be produced from glyoxylate by the enzyme D-glycerate dehydrogenase. This amino acid is found in higher concentrations in the spinal cord than the rest of the CNS. Glycine acts as an inhibitory neurotransmitter predominantly in the brainstem and spinal cord (Nestler et al., 2001). As discussed above, a very important role that glycine also plays is to augment the NMDA mediated frequency of NMDA-receptor channel opening. This effect is strychnine-insensitive and pharmacologically suggests that the actions of glycine on NMDA receptor function are different from that of the effects on the spinal cord where glycine’s inhibitory effect is blocked by strychnine (Cooper et al., 2001). The allosteric modulation of NMDA receptors via a glycine-insensitive site is further underscored by receptor binding experiments yielding an anatomic distribution similar to that of NMDA receptors. Functionally, it has been postulated that glycine is also able to augment the NMDA mediated responses by speeding up the recovery process of the receptor (Cooper et al., 2001).

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