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The Coupling of Neurotransmitter Receptors to Ion Channels in the Brain

ROGER A. NICOLL

Recent studies on the action of neurotransmitters on hippocampal pyramidal cells indicate that different neurotransmitter receptors that use either the same or different coupling mechanisms converge onto the same ion channel. Conversely, virtually all of the neurotransmitters act on at least two distinct receptor subtypes coupled to different ion channels on the same cell. The existence of both convergence and divergence in the action of neurotransmitters results in a remarkable diversity in neuronal signaling.

IN THE 1950S THE USE OF INTRACELLULAR RECORDING ESTABlished the chemical nature of synaptic transmission in the mammalian central nervous system (*1*). Specifically, neurotransmitters produce their excitatory and inhibitory effects on neurons by rapidly and briefly increasing membrane ion permeability. Over the past 25 years, the use of iontophoretic techniques, in which drugs can be directly applied onto single neurons in vivo, allowed such compounds as γ-aminobutyric acid (GABA), glycine, glutamate, norepinephrine, acetylcholine (ACh), and serotonin to be identified as neurotransmitters in the mammalian brain (*2*). However, investigators had to turn to simpler invertebrate preparations for more detailed studies of the mechanisms involved in the action of neurotransmitters. These preparations, as well as preparations of peripheral ganglia from vertebrates, avoided the numerous constraints of in vivo mammalian preparations, such as the complexity of the neuronal circuitry, barriers to drug delivery, and lack of stability for intracellular recording. Investigators were thus able to demonstrate an extraordinary diversity in neurotransmitter action (*3*). The results to be discussed in this review will demonstrate that studies that could originally only be done in these invertebrate in vitro preparations can now be performed in in vitro slice preparations of the mammalian brain. The slice preparation has the advantage that fully differentiated adult brain tissue, in which the local neuronal circuitry is often intact and functioning, is used.

Although biochemical studies on brain slices have been carried out for over half a century, electrical recordings were first made in the mid-1960s by Yamamoto (*4*). The development of the transverse hippocampal slice in which all of the local circuits can be functionally preserved (*5*), coupled with the demonstration that high quality intracellular recording could routinely be obtained from these slices (*6*), led to a rapid explosion in the use of brain slice preparations for electrophysiological analysis (*7*).

Previous results by neurochemists suggested a functional role for a variety of putative neurotransmitters. For instance, in the hippocampus, neurochemical and immunohistochemical studies have found close to 20 different putative neurotransmitters in nerve cell bodies or fibers or both. Specific receptor binding sites for many of these same compounds have been identified and characterized (*8*). In addition, the brain has long provided the richest source of tissue for biochemists studying various components of second messenger systems (*9*), which mediate the effects of many neurotransmitters.

I have focused my attention on the hippocampal slice preparation for a number of reasons. There are many neurotransmitter candidates in this cortical structure (Table 1). In addition, the precise laminar organization of cellular components permits much of the local circuitry to remain intact and greatly facilitates intracellular recording and selective stimulation of afferent neuronal pathways. Finally, the hippocampus is of considerable interest in its own right, because it plays a key role in memory (*10*) and epilepsy (*11*).

Neurotransmitters exert their effects by opening or closing ion channels in the neuronal membrane, either directly or through the activation of intermediate proteins. Until recently, it was believed that most neurotransmitters produced a simple hyperpolarization or depolarization of brief duration in the postsynaptic membrane. However, it is now known that neurotransmitters can alter, over a longer time span, the properties of voltage-dependent ionic conductances that are involved in the control of cell excitability (*3*). In particular, a wide range of K^+ currents and Ca^{2+} currents are modulated by neurotransmitters, resulting in such changes as increased action potential duration, changes in firing frequency and firing pattern, and increased Ca^{2+} entry during an action potential. Thus, the modulation of voltage-dependent ion channels allows for a finer control via multiple mechanisms of various aspects of cell excitability.

The author is a professor in the Departments of Pharmacology and Physiology, School of Medicine, University of California, San Francisco, CA 94143.

This review will focus on recent experiments in the hippocampus, with reference to work in other areas of the brain. I will discuss the Ca^{2+}-dependent K^+ currents, which play an important role in controlling pyramidal cell excitability, because many neurotransmitters modulate this current. I will then give some recent examples of modulation of various other K^+ conductances, which control different aspects of pyramidal cell excitability, focusing on the actions of norepinephrine, ACh, GABA, and serotonin. I will conclude by putting forward some possible unifying concepts that emerge from this work.

Calcium-Dependent Control of Cell Excitability

Hippocampal pyramidal cells have two distinct K^+ conductances that are activated by a rise in intracellular Ca^{2+} and generate a slow afterhyperpolarization (AHP) and a fast AHP. The slow AHP is elicited by a series of action potentials and is dependent on the influx of Ca^{2+} across the membrane (*12*). It is found in a variety of neurons in the central nervous system. The slow AHP plays a major role in the slowing of action potential discharge rate (accommodation or adaptation) that typically occurs during a long duration depolarization (*13*). Thus if the slow AHP is blocked, either by blocking the rise in intracellular Ca^{2+} with the intracellular injection of a chelator of Ca^{2+} (Fig. $1A_1$) or by blocking Ca^{2+} entry through voltage-dependent Ca^{2+} channels (Fig. $1A_2$), many more action potentials are evoked by the same depolarizing current pulse.

The entry of Ca^{2+} also activates a fast AHP, which is important in action potential repolarization (*14, 15*). A similar fast AHP has been identified and characterized in bullfrog sympathetic ganglion cells (*16*). In hippocampal pyramidal cells, the fast AHP, unlike the slow AHP, is blocked by the scorpion toxin, charybdotoxin, suggesting that the most likely channel candidate for the fast AHP is the large conductance, voltage-dependent, Ca^{2+}-activated K^+ channel described in these (*17*) and many other cells (*18*).

In summary, in hippocampal pyramidal cells there are two distinct Ca^{2+}-activated K^+ conductances: a fast one which repolarizes the action potential and a slow one responsible for spike frequency adaptation or accommodation.

Analysis of Neurotransmitter Mechanisms

Norepinephrine. Hippocampal pyramidal cells receive a clearly defined noradrenergic input from the locus ceruleus (*19*). Previous studies have reported primarily an inhibitory action of norepinephrine (NE) on these cells (*20*). Although in our slice experiments (*21*) application of NE evoked a hyperpolarization, this effect on the membrane potential is small and variable. In contrast, we found that responses to iontophoretically applied glutamate were dramatically enhanced by NE (Fig. $1B_1$). A similar increase in excitability caused by NE could be demonstrated when cells were excited with depolarizing current pulses (Fig. $1B_2$). Close inspection of the action of NE indicates that it prevents the accommodation of spike discharge that

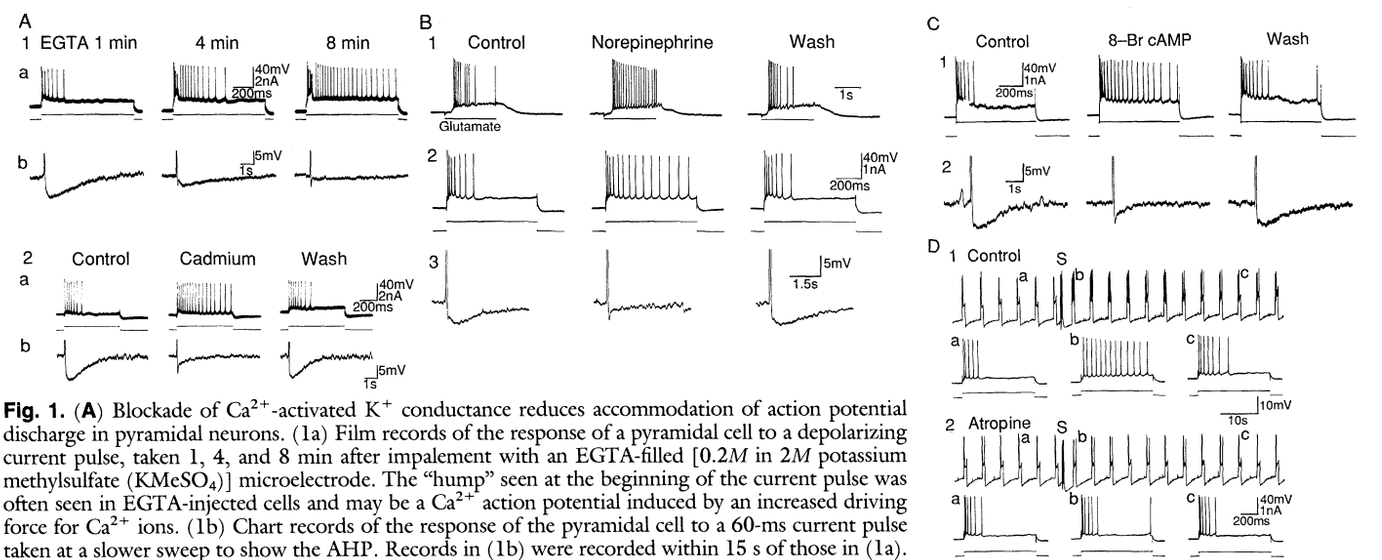

Fig. 1. (**A**) Blockade of Ca^{2+}-activated K^+ conductance reduces accommodation of action potential discharge in pyramidal neurons. (1a) Film records of the response of a pyramidal cell to a depolarizing current pulse, taken 1, 4, and 8 min after impalement with an EGTA-filled [0.2*M* in 2*M* potassium methylsulfate ($KMeSO_4$)] microelectrode. The "hump" seen at the beginning of the current pulse was often seen in EGTA-injected cells and may be a Ca^{2+} action potential induced by an increased driving force for Ca^{2+} ions. (1b) Chart records of the response of the pyramidal cell to a 60-ms current pulse taken at a slower sweep to show the AHP. Records in (1b) were recorded within 15 s of those in (1a). Membrane potential, −55 mV. (2a) Film records of the response of a pyramidal cell to a depolarizing current pulse in control, after 14 min in 100 μ*M* cadmium, and 80 min after return to control solution. (2b) Chart records of the response of the cell to a short (60-ms) current pulse, recorded within 15 s of the records in (2a). Membrane potential, −57 mV. [Reprinted from (*13*) with permission, © 1984] (**B**) Norepinephrine blocks the adaptation of action potential discharge and the Ca^{2+}-activated K^+ response in a CA1 hippocampal pyramidal cell. (1) Film records of response to 30 nA of ionophoretically applied glutamate. (2) Film records of response of cell to a depolarizing current pulse. The current trace is below the voltage trace. (3) Chart records of response to a 100-ms depolarizing current pulse. The responses in (1) to (3) in the middle column were obtained approximately 4 min after the addition of 10 μ*M* NE. The records in "Wash" were obtained approximately 30 min after returning to the control solution. Membrane potential, −58 mV. The gain in (1) is the same as in (2). [Reprinted from (*21*) with permission, © 1982, Macmillan Magazines, Ltd.] (**C**) 8-Br cAMP reduces accommodation in hippocampal CA1 pyramidal cells. All records taken from the same pyramidal cell bathed in normal medium. (1) Photographic records of the response of a pyramidal cell to a long (700-ms) depolarizing current pulse that elicits an accommodating train of action potentials. (2) Chart records of the response of the cell to a short duration (70 ms) depolarizing current stimulus that elicits an AHP. Bath application of 8-Br cAMP (1 m*M* for 10 min) caused a reduction in the slow component of the AHP and in accommodation pari passu. The effects of 8-Br cAMP were reversed 20 min after removing the drug from the superfusate. Membrane potential, −61 mV. [Reprinted from (*26*) with permission, © 1986] (**D**) Electrical stimulation in stratum oriens blocks accommodation of cell discharge in CA1 pyramidal cells. (1) Upper trace is a chart record showing long (800-ms) depolarizing pulses applied every 5 s. Traces below are simultaneously recorded film records of the 800-ms pulse showing accommodation of cell firing. Current monitor is displayed immediately below. a, b, and c in upper and lower records correspond to the same 800-ms pulses (a) before, (b) immediately after, and (c) 90 s after the train of stimuli (40 Hz for 0.5 s; S above trace). (2) same experiment as in (1), but with atropine (1 μ*M*) added to the superfusate. The block of accommodation and increased cell firing seen in (1b) after stimulus is completely antagonized by atropine (2b). Calibration in (1) is for both chart records; calibration in (2) is for all film records. Membrane potential, −68 mV. [Reprinted from (*32*) with permission, © 1984]

Table 1. Neurotransmitter candidates in hippocampus.

Glutamate	Enkephalin
Aspartate	Dynorphin
GABA	Angiotensin
Acetylcholine	Somatostatin
Norepinephrine	Cholecystokinin
Serotonin	Vasoactive intestinal polypeptide
Adenosine	Galanin
Histamine	Neuropeptide Y
Dopamine	Substance P
	Corticotropin-releasing factor

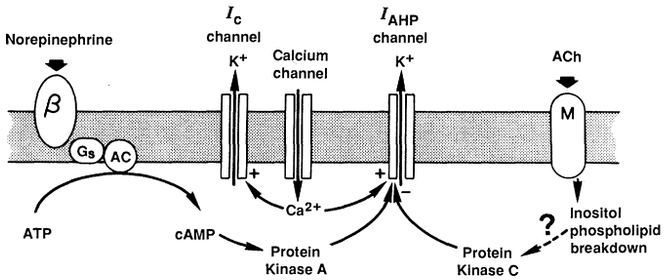

Fig. 2. Diagram of the proposed mechanisms of action of norepinephrine and acetylcholine in blocking the slow Ca^{2+}-activated K^+ conductance.

normally occurs during the depolarization and does this by blocking the slow AHP (Fig. $1B_3$). This action on the slow AHP has been confirmed by others (*22–24*), and a similar blockade has been reported for neocortical pyramidal cells (*25*). This effect is selective since there is no change in action potential duration or the fast AHP, although a reduction in the A current has been reported (*24*).

Pharmacological studies have shown that this action of NE is mediated by a β_1 adrenergic receptor. The β adrenergic receptors in pyramidal cells seem to exert their effects by activating adenylate cyclase (*26*). First, the action of NE is mimicked by the membrane-permeant analog of adenosine 3′,5′-monophosphate (cAMP) 8-bromo cyclic AMP (8-Br cAMP) (Fig. 1C), as well as by forskolin, a direct activator of adenylate cyclase. Second, blocking of phosphodiesterase activity either with 3-isobutyl-1-methylxanthine or Ro20-1724 potentiates the action of NE. Finally, reducing adenylate cyclase activity with SQ22,536 reduces the action of NE. Because cAMP has no effect on Ca^{2+} action potentials, we have proposed that the blockade, which presumably involves cAMP-dependent protein kinase, occurs at a step subsequent to the entry of Ca^{2+}.

Thus, NE modulates the Ca^{2+}-activated K^+ conductance responsible for slowing repetitive spiking, and this effect appears to be mediated through the production of the second messenger cAMP. Histamine acting on H_2 receptors (*22*) and corticotropin-releasing factor (*27*) also block the same Ca^{2+}-activated K^+ conductance. Because both of these putative neurotransmitters can elevate cAMP levels, their actions are also probably mediated through cAMP.

Acetylcholine. The dense cholinergic input to hippocampal pyramidal cells is derived from neurons in the medial septal nucleus (*28*). Although ACh strongly excites virtually all pyramidal cells (*29*), this excitatory action is quite complex and involves the blockade of at least four distinct K^+ currents (*30–34*). All of these actions are mediated by muscarinic receptors, but it is not yet clear whether these multiple actions are mediated through a single transduction mechanism. One of the most potent actions of ACh, or of stable analogs such as carbachol, is a blockade of the slow AHP (*31–33*), which has also been observed in neocortical neurons (*25, 35*). As with NE, this blockade is associated with a loss in adaptation of spike discharge, but there is no effect of ACh on the fast AHP or action potential duration (*15*). We have been able to mimic the effects of exogenously applied cholinergic agonists by electrical stimulation of the cholinergic afferents in the slice (*32, 33*). Thus, a brief tetanus can cause a prolonged atropine-sensitive block in accommodation (Fig. 1D). Muscarinic receptor activation can reduce Ca^{2+} currents in CA3 pyramidal cells (*36*). It is unclear if a similar action occurs in CA1 pyramidal cells, because one would expect carbachol to reduce the fast AHP if this were the case. It is most unlikely that the blockade of the slow AHP by ACh is also mediated through cAMP because muscarinic receptors are negatively coupled to adenylate cyclase. However, muscarinic receptor activation in the hippocampus results in a large increase in phosphotidylinositol (PI) turnover (*37*). This raises the possibility that one of the breakdown products, inositol trisphosphate (IP_3) or diacylglycerol, might mediate the effects of ACh. Diacylglycerol is an endogenous activator of protein kinase C, which in turn phosphorylates a variety of substrate proteins. To test for the possible involvement of this kinase, phorbol esters, which potently activate this enzyme, were applied and found to block the AHP (*38, 39*). On the other hand, analogs of phorbol esters that are incapable of activating protein kinase C have no effect on the slow AHP. These findings are consistent with the proposal that activation of protein kinase C mediates the effects of muscarinic receptor activation. However, the muscarine analog, oxotremorine, which only weakly activates PI turnover (*37*), is as potent as carbachol at blocking the AHP (*40*). This observation does not entirely rule out the involvement of PI turnover in the blockade of the AHP, because it is possible that considerable receptor reserve exists for this action or that diacylglycerol is produced by other metabolic pathways not linked to IP_3 generation (*41*).

Our working hypothesis for the action of NE and ACh on the slow AHP is diagrammed in Fig. 2. In the center of the figure is a voltage-dependent Ca^{2+} channel. Entry of Ca^{2+} through this channel results in the activation of two distinct Ca^{2+}-activated K^+ channels. The I_C channel, which is the large conductance, voltage-dependent, Ca^{2+}-activated K^+ channel, repolarizes the action potential. This channel does not appear to be affected by neurotransmitters. Due to the voltage sensitivity of this channel, it closes rapidly when the membrane repolarizes despite the continued presence of Ca^{2+}. Internal Ca^{2+} also activates a second class of K^+ channels, which is responsible for the slow AHP (I_{AHP}) and controls the repetitive firing of pyramidal cells. This conductance is under the control of at least two second messenger systems. The left-hand side of the diagram shows a β adrenergic receptor, which when occupied catalyzes the formation of cAMP via the stimulatory guanosine triphosphate–binding protein (G_s), which activates adenylate cyclase (AC). Cyclic AMP, presumably acting through cAMP-dependent protein kinase (protein kinase A), blocks the slow AHP, possibly by phosphorylating the I_{AHP} channel itself. Muscarinic receptor activation, as well as activation of protein kinase C, also blocks the slow AHP. These results raise the intriguing possibility that two second messenger systems may phosphorylate the same channel protein. In this regard cAMP-dependent protein kinase and protein kinase C can in some cases phosphorylate the same protein (*42*). Interestingly, it has recently been proposed that the S channel in *Aplysia* sensory cells can be modulated in opposite directions by two distinct second messenger systems (*43*).

Another K^+ current in hippocampal pyramidal cells that is blocked by muscarinic receptor activation is the M current, a time- and voltage-dependent K^+ current (*30, 33*). This current turns on slowly with membrane depolarization and, in sympathetic neurons, contributes to the accommodation of action potential discharge (*44*). Muscarinic agonists that potently increase PI turnover block the M current. In contrast, weak agonists do not affect the M current and actually antagonize the action of strong agonists (*40*). Although

these results implicate PI turnover in the blockade of the M current, activation of protein kinase C by phorbol esters fails to block the M current (*39, 40*). This suggests that diacylglycerol released during PI breakdown does not mediate blockade of the M current, but instead that IP_3 may be involved. We therefore examined the effect of intracellular injections of IP_3 on the M current. Although these experiments are technically difficult, we found that IP_3 did reduce the M current (*40*). The most clearly established role for IP_3 is its ability to release Ca^{2+} from intracellular stores (*45*). Although involvement of Ca^{2+} cannot be entirely excluded, blockade of the M current by muscarinic agonists is unimpaired by buffering intracellular Ca^{2+} (*40*). The effects of activating PI breakdown in response to muscarinic receptor stimulation thus may include attenuation of both I_{AHP} and the M current. These currents are likely to act synergistically to block accommodation (*44*). Quite different results have been reported for the M current in sympathetic ganglia where IP_3 injections have little effect, whereas phorbol esters cause a partial block of the M current (*46*).

GABA. The GABAergic input to pyramidal cells originates from intrinsic local circuit neurons (*47*). Local iontophoretic application of GABA onto pyramidal cells evokes a variety of responses that depend critically on the location of the pipette, as well as on the amount of GABA ejected (*48–51*). When the pipette is positioned very close to the soma of a pyramidal cell, small amounts of iontophoretically ejected GABA produces a pure hyperpolarization associated with a large increase in membrane conductance (Fig. 3, A_{1a} and A_{1b}). The reversal potential for this action is approximately −70 mV. This hyperpolarizing action is entirely blocked by $GABA_A$ antagonists such as bicuculline (Fig. $3A_{1c}$) and picrotoxin and results from an increase in Cl^- conductance, since shifting the Cl^- gradient across the membrane shifts the reversal potential for this response. The response can be mimicked by such $GABA_A$ agonists as muscimol and THIP (4,5,6,7-tetrahydroisoxazolo [5,4-c] pyridin-3-ol). If one increases the amount of GABA ejected at the soma or positions the pipette in the dendritic field, a depolarizing component to the GABA response appears. When applied in the dendritic region, the depolarizing response predominates and is associated with a large increase in membrane conductance (Fig. $3A_{1b}$). The evidence that this response may also involve an increase in Cl^- conductance is based on the following observations. First, the response is blocked by $GABA_A$ antagonists (Fig. 3) and $GABA_A$ receptors are usually coupled to a Cl^- conductance mechanism. However, $GABA_A$ agonists such as muscimol and THIP are not as effective at eliciting this response than GABA, raising the possibility that some differences in $GABA_A$ receptors might exist (*49*). Second, the reversal potential for the responses shifts when the concentration of Cl^- in the extracellular medium is reduced (*49, 50*). Third, furosemide, which blocks Cl^- transport in other systems, blocks both the dendritic depolarizing responses and the somatic hyperpolarizing responses but does not alter the underlying increase in membrane conductance (*51*).

The GABA-induced depolarization in the dendritic region is often followed by a hyperpolarization when higher amounts of GABA are ejected (Fig. 3A). This hyperpolarization is resistant to $GABA_A$ antagonists and can be studied in relative isolation after blockade of the depolarizing component (Fig. 3, A_{1c} and A_{2c}) (*49, 50*). It is associated with an increase in membrane conductance and has a reversal potential of about −90 mV in a superfusing medium containing 5.4 m*M* K^+. Increasing the extracellular concentration of K^+ causes a depolarizing shift in the reversal potential, whereas shifting the Cl^- gradient has little effect on the hyperpolarization. Thus it can be concluded that GABA can open K^+ channels by a bicuculline-resistant action. This action can be mimicked by the selective $GABA_B$ agonist, baclofen (Fig. 3, A_{2b} and A_{2c}) (*49, 52*). As would be expected, baclofen responses are unaffected by $GABA_A$ receptor antagonists (Fig. $3A_{2c}$). In addition, in the presence of

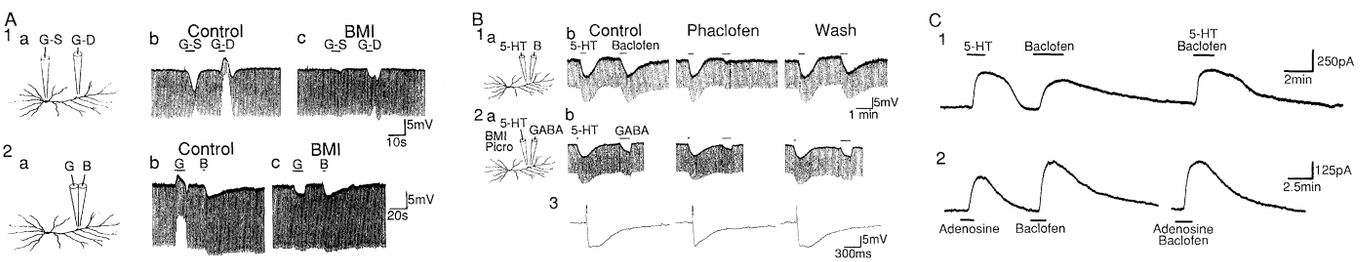

Fig. 3. (**A**) Actions of GABA on hippocampal pyramidal cells. (1a) Diagram of placement of iontophoretic electrodes. (1, b and c) Somatic (G-S) and dendritic (G-D) GABA applications were repeated after superfusion with bicuculline methiodide (BMI) (100 μ*M*) for 20 min. Downward deflections represent the response to constant-current hyperpolarizing pulses applied throughout the recording. (2a) Diagram of placement of double-barreled iontophoretic electrode. (2, b and c) Responses to GABA (G) (60 nA) and baclofen (B) (70 nA) before (2b) and 25 min after superfusing BMI (100 μ*M*) (2c). Membrane potentials, −55(1) and −62 mV(2). [Reprinted from (*50*) with permission, © 1985] (**B**) Selective antagonism by phaclofen of $GABA_B$ responses and the slow IPSP in CA1 pyramidal cells. (1) The serotonin (5-HT) and baclofen (B) electrodes were positioned at the surface of stratum radiatum, 200 μm away from the pyramidal cell layer (1a). 5-HT (110 nA) and baclofen (120 nA) iontophoresed from these electrodes induce hyperpolarizing responses as shown by intracellular recording from the pyramidal cell (1b, control trace). In the presence of phaclofen (0.5 m*M*), superfused for 6 min, the baclofen response is strongly antagonized. In striking contrast, the 5-HT response remains unchanged. The baclofen response recovers 6 min after washing phaclofen from the bath. (2) GABA and 5-HT are iontophoresed to the dendrites of the pyramidal cell in the presence of the $GABA_A$ antagonists bicuculline methiodide (BMI) (40 μ*M*) and picrotoxin (Picro) (20 μ*M*) (2a). Intracellular recordings from the pyramidal cell show the hyperpolarizing responses induced by GABA (120 nA) and 5-HT (100 nA) (2b, control trace). In the presence of phaclofen (0.5 m*M*) superfused for 15 min, the bicuculline-resistant GABA response is depressed. In contrast, the 5-HT response is unchanged. The GABA response recovers 20 min after washing phaclofen from the bath. (1) and (2) are recordings from two different cells. Membrane potential, −62 mV (1); −68 mV (2). The drugs were iontophoresed for the periods indicated by the bars. Calibration in (1b) applies also to (2b). (3) Synaptically evoked potential induced by orthodromic stimulation in stratum radiatum was recorded from a CA1 pyramidal cell. As shown in the control trace, the electrical stimulation evokes, following the excitatory synaptic potential (truncated in this record), a biphasic hyperpolarizing response including a fast and a slow IPSP. In the presence of phaclofen (0.2 m*M*) superfused for 5 min, the slow IPSP is abolished (middle trace). In contrast, the fast IPSP remains unaffected. The slow IPSP recovers 15 min after washing the phaclofen from the bath. Resting potential, −56 mV. [Reprinted from (*53*) with permission, © 1988, Macmillan Magazines Ltd.] (**C**) 5-HT, baclofen, and adenosine hyperpolarize pyramidal cells through a common conductance mechanism. (1) Continuous voltage-clamp record of the outward currents evoked by 5-HT (30 n*M*) and baclofen (30 μ*M*) applied in the bath alone or together. [Reprinted from (*60*) with permission, © 1986, AAAS] (2) Voltage-clamp records of the outward currents evoked by adenosine (100 μ*M*) and baclofen (30 μ*M*) applied in the bath alone or together (*61*). The responses evoked by the combined application of the two drugs in (1) and (2) are no longer than that evoked by 5-HT (1) or baclofen (2) alone.

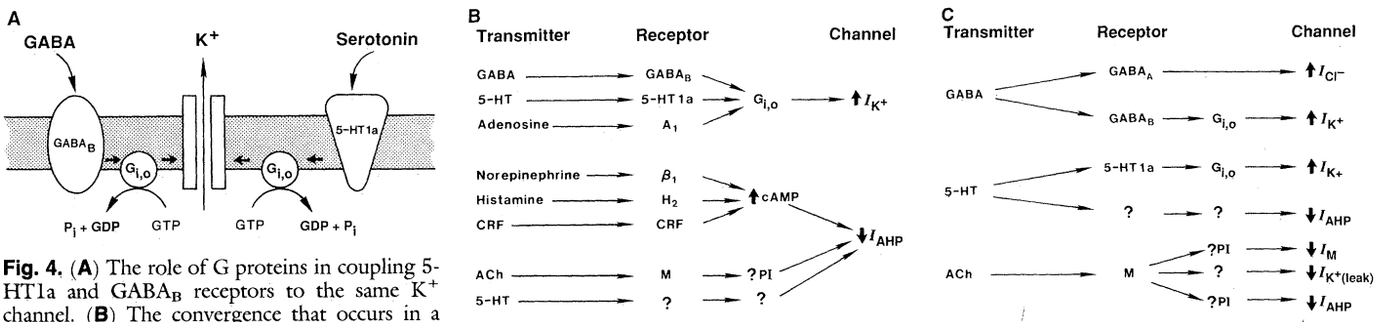

Fig. 4. (**A**) The role of G proteins in coupling 5-HT1a and $GABA_B$ receptors to the same K^+ channel. (**B**) The convergence that occurs in a single cell between neurotransmitter receptors and the ion channel that is modulated. (**C**) The divergence that occurs in a single cell when a single neurotransmitter acts on multiple receptors.

$GABA_A$ antagonists, the outward current evoked by brief iontophoretic applications of GABA to the dendrites is essentially completely occluded by bath application of a high concentration of baclofen, suggesting that the two compounds activate the same K^+ conductance (*53*).

Stimulation of the axons of pyramidal cells or weak stimulation of afferent fibers results in a fast inhibitory postsynaptic potential (IPSP) that peaks at approximately 50 ms and lasts for 200 to 300 ms. This IPSP is blocked by $GABA_A$ antagonists, has a reversal potential of approximately −70 mV (*54*), and is dependent on the Cl^- gradient across the cell membrane. Stronger orthodromic stimulation evokes a slower and later component that peaks at about 200 ms and lasts about 1 s (*55*). This response is referred to variously as the slow or late IPSP or the late hyperpolarizing potential (LHP). It is resistant to $GABA_A$ antagonists and has a reversal potential of approximately −90 mV. These features, which are identical to the $GABA_B$ receptor–mediated action of GABA, suggest that GABA may be the transmitter responsible for the slow IPSP. However, mimicry of action is not sufficient for transmitter identification. For instance, serotonin (see below) also mimics the slow IPSP. The phosphonic acid derivative of baclofen, phaclofen, is a baclofen antagonist on spinal cord and gut neurons (*56*). We have found (*53*) that phaclofen is a selective antagonist of baclofen (Fig. $3B_1$) and the bicuculline-resistant action of GABA (Fig. $3B_2$) on pyramidal cells. In addition, it blocks the slow IPSP but has no effect on the fast IPSP (Fig. $3B_3$).

These results suggest that GABA released from interneurons interacts with two subtypes of GABA receptors on the membrane of pyramidal cells—the $GABA_A$ receptor, which generates the fast IPSP, and the $GABA_B$ receptor, which generates the slow IPSP. Weak afferent inputs evoke only the fast IPSP, presumably because the interneurons do not fire a sufficient number of action potentials to release enough GABA to activate the $GABA_B$ receptors, which require higher concentrations of GABA for their activation. Recently (*57*) it has been found that phaclofen blocks slow IPSPs in the dorsolateral septal nucleus, the dorsal lateral geniculate nucleus, and the neocortex, strongly suggesting that the role of $GABA_B$ receptors in postsynaptic inhibition is widespread.

Serotonin. The serotonergic input to pyramidal cells originates from the midbrain raphe nucleus (*58*). Until quite recently serotonin was thought to have a simple inhibitory action on pyramidal cells, involving a hyperpolarization due to the opening of K^+ channels (*59*). Superficially, this response, as well as the response due to activating $GABA_B$ receptors (see above), resembles a classical neurotransmitter action in which binding to the receptor results in the rapid opening of a channel intimately associated with the receptor molecule. However, this coupling mechanism may also be indirect. We have found that, when a maximal response has been evoked with serotonin, the $GABA_B$ receptor agonist baclofen was unable to produce any additional response (Fig. $3C_1$) (*60*). This finding indicates that the two receptors share the same channels. If the two receptors were each to have their own channels, then the responses evoked by the two receptors should have been additive. We have found that a similar occlusion occurs between adenosine and baclofen responses (Fig. $3C_2$) (*61*).

We have analyzed the basis for this sharing of K^+ channels (*60*). Based on a number of observations we have excluded the possibility that the linkage occurs through such diffusible second messengers as cAMP, Ca^{2+}, or diacylglycerol. However, our experiments with pertussis toxin and stable guanosine diphosphate (GDP) and guanosine triphosphate (GTP) analogs implicate a GTP-binding (G) protein in these responses (*60*). Prior treatment of the hippocampus with pertussis toxin, which is known to ADP (adenosine diphosphate)-ribosylate and inactivate several G proteins, blocked the action of baclofen and serotonin entirely. On the other hand, the stable GTP analog, guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS), which maintains the G protein in its activated state, mimics the action of the two receptors. Whole-cell recording experiments from cultured hippocampal neurons (*62*) provide similar results, but also provide additional convincing evidence for the involvement of a G protein. It was found that adenosine and serotonin responses can be dialyzed away in these recordings due to the rapid exchange between the cytoplasm and the solution in the recording electrode, but, if GTP is added to the patch pipette solution, the responses remain quite stable. Thus it appears that a pertussis toxin–sensitive G protein couples serotonin, $GABA_B$, and adenosine receptors to the same K^+ channel. A similar coupling mechanism has been reported for α_2 adrenergic receptors and opiate receptors in locus ceruleus neurons (*63*) and $GABA_B$ and dopamine D_2 receptors in substantia nigra neurons (*64*).

Because we were unable to link a number of the known diffusible second messenger systems to this coupling mechanism, we have suggested that the G protein may directly couple the receptors to the K^+ channels (Fig. 4A). Although the G protein has been provisionally labeled as either G_i or G_o, the results do not exclude some other pertussis toxin–sensitive G protein. A similar mechanism has been shown for the muscarinic inhibition of K^+ channels in cardiac tissue (*65*). It should be pointed out that our results do not exclude a role of arachidonic acid metabolites in mediating serotonin, baclofen, and adenosine responses. Such a mechanism has recently been proposed for the actions of the peptide FMRFamide in *Aplysia* sensory cells (*66*).

Interestingly, activation of protein kinase C with phorbol esters blocks serotonin and baclofen responses (*60*) as well as adenosine responses (*67*). Because protein kinase C has been shown to phosphorylate and inactivate certain pertussis toxin–sensitive G proteins (*68*), this kinase may well block the responses by blocking the G protein. Such an action may have physiological relevance, since muscarinic receptor activation has also been reported to reduce the responses to baclofen and adenosine (*67*).

In addition to evoking the hyperpolarization, which is mediated by serotonin 1A (5-HT1a) receptors (*69*), serotonin also evokes a long-lasting depolarization that follows the hyperpolarization (*69*). This depolarization is due to a decrease in a resting K^+ conductance and is also associated with a block in the AHP. This action on the AHP occurs in the absence of any reduction in Ca^{2+} action potentials. The pharmacology of the depolarization and blockade of the AHP does not conform to any of the sites that have so far been characterized in binding studies. The coupling mechanisms involved in these actions are also unclear but do not appear to involve cAMP. As expected, the blockade of the AHP by serotonin reduces action potential frequency adaptation, which counteracts the inhibitory action of serotonin on 5-HT1a receptors and outlasts the inhibitory action. These excitatory actions are remarkably similar to the slow excitatory actions of serotonin in neurons of the gut (*70*) and may be related to excitatory responses to serotonin in other regions of the brain (*71*).

Conclusions

Hippocampal pyramidal cells respond to a far greater number of neurotransmitters than there are known transduction mechanisms. Thus a number of receptor types modulate the same ion channel, either by sharing the same second messenger system or by using separate systems (Fig. 4B). GABA, serotonin, and adenosine, each acting on distinct receptors, activate a G protein, which opens a common K^+ channel (Fig. 4B). Norepinephrine, histamine, and corticotropin-releasing factor, all probably acting via cAMP, block the K^+ channel responsible for the AHP (Fig. 4B). In addition both ACh and serotonin block the same channels but are unlikely to use cAMP. Turnover of PI and subsequent activation of protein kinase C may be involved in the action of ACh.

In contrast to this striking convergence of neurotransmitter receptors onto the same ion channel, we have found that each pyramidal cell typically has more than one type of receptor for each neurotransmitter that is coupled to different ion channels (Fig. 4C). The existence of multiple receptor subtypes on a single postsynaptic cell allows considerable complexity in neuronal signaling. First, the nature of the postsynaptic response to a neurotransmitter can depend on the amount of transmitter released. For instance, the $GABA_A$ receptor can be activated at lower concentrations of GABA than can the $GABA_B$ receptor. Thus the amount of GABA released will determine which receptor subtypes will be activated. Second, the type of receptor and the coupling mechanism will determine the duration of the postsynaptic response. Thus receptors in which the ion channel is an integral part of the receptor protein, as is the case for the $GABA_A$ receptor, evoke responses that last, at most, tens of milliseconds. Receptors that are coupled to G proteins, such as the $GABA_B$ receptor, evoke responses that last hundreds of milliseconds. Finally, receptors that are coupled to diffusible second messengers evoke responses that last for at least tens of seconds. Thus when one neurotransmitter acts on two receptor subtypes, a temporal separation in responses can occur. It is also possible, although we have no experimental evidence on this point, that different subtypes might be segregated to different parts of the neuron and might be activated by different synapses.

These results thus emphasize that synaptic transmission in the central nervous system is considerably more complex than we previously thought. The large number of neurotransmitters that converge onto single neurons and the large number of transduction mechanisms in single neurons offer a wide variety of mechanisms for information processing.

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Research Articles

Parallel Stranded DNA

JOHAN H. VAN DE SANDE, NIELS B. RAMSING, MARKUS W. GERMANN, WIM ELHORST, BERND W. KALISCH, EBERHARD V. KITZING, RICHARD T. PON, ROBERT C. CLEGG, THOMAS M. JOVIN

A series of four hairpin deoxyoligonucleotides was synthesized with a four-nucleotide central loop (either C or G) flanked by the complementary sequences $d(T)_{10}$ and $d(A)_{10}$. Two of the molecules contain either a 3′-p-3′ or 5′-p-5′ linkage in the loop, so that the strands in the stem have the same, that is, parallel (ps) polarity. The pair of reference oligonucleotides have normal phosphodiester linkages throughout and antiparallel (aps) stem regions. All the molecules adopt a duplex helical structure in that (i) the electrophoretic mobilities in polyacrylamide gels of the ps and aps oligomers are similar. (ii) The ps hairpins are substrates for T4 polynucleotide kinase, T4 DNA ligase, and *Escherichia coli* exonuclease III. (iii) Salt-dependent thermal transitions are observed for all hairpins, but the ps molecules denature 10°C lower than the corresponding aps oligomers. (iv) The ultraviolet absorption and circular dichroism spectra are indicative of a base-paired duplex in the stems of the ps hairpins but differ systematically from those of the aps counterparts. (v) The bis-benzimidazole drug Hoechst-33258, which binds in the minor groove of B-DNA, exhibits very little fluorescence in the presence of the ps hairpins but a normal, enhanced emission with the aps oligonucleotides. In contrast, the intercalator ethidium bromide forms a strongly fluorescent complex with all hairpins, the intensity of which is even higher for the ps species. (vi) The pattern of chemical methylation is the same for both the ps and aps hairpins. The combined results are consistent with the prediction from force field analysis of a parallel stranded right-handed helical form of $d(A)_n \cdot d(T)_n$ with a secondary structure involving reverse Watson-Crick base pairs and a stability not significantly different from that of the B-DNA double helix. Models of the various hairpins optimized with force field calculations are described.

THE POLYMORPHIC NATURE OF DOUBLE-STRANDED DNA IS well established (*1*, *2*). A common feature of the three major families of A-, B-, and Z-DNA duplexes is the antiparallel disposition of the constituent strands (*2*, *3*). Single crystal diffraction analysis of oligonucleotide duplexes indicates that only Watson-Crick base pairing occurs in the structures examined to date (*3*). However, alternative conformations are feasible. Thus, both experimental evidence and model calculations support the existence of parallel stranded double helices stabilized by (i) hemiprotonation of

J. H. van de Sande, M. W. Germann, B. W. Kalisch, and R. T. Pon are at the Department of Medical Biochemistry, University of Calgary, Calgary, Alberta, Canada T2N 4N1. N. B. Ramsing, W. Elhorst, E. v. Kitzing, R. C. Clegg, and T. M. Jovin are at the Department of Molecular Biology, Max Planck Institute for Biophysical Chemistry, 3400 Göttingen, Federal Republic of Germany.