Convergence and divergence of neurotransmitter action in human cerebral cortex

(David A. McCormick and Anne Williamson)

ABSTRACT The postsynaptic actions of acetylcholine, adenosine, γ-aminobutyric acid, histamine, norepinephrine, and serotonin were analyzed in human cortical pyramidal cells maintained in vitro. The actions of these six putative neurotransmitters converged onto three distinct potassium currents. Application of acetylcholine, histamine, norepinephrine, or serotonin all increased spiking by reducing spike-frequency adaptation, in part by reducing the current that underlies the slow afterhyperpolarization. In addition, application of muscarinic receptor agonists to all neurons or of serotonin to middle-layer cells substantially reduced or blocked the M-current (a K+ current that is voltage and time dependent). Inhibition of neuronal firing was elicited by adenosine, baclofen (a γ-aminobutyric acid type B receptor agonist), or serotonin and appeared to be due to an increase in the same potassium current by all three agents. These data reveal that individual neuronal currents in the human cerebral cortex are under the control of several putative neurotransmitters and that each neurotransmitter may exhibit more than one postsynaptic action. The specific anatomical connections of these various neurotransmitter systems, as well as their heterogeneous distribution of postsynaptic receptors and responses, allows each to make a specific contribution to the modulation of cortical activity.

Convergence and divergence of neurotransmitter action has important implications for understanding functional systems in the brain. Not only do the neuronal systems underlying behavior consist of a number of transmitter pathways, but each pathway is involved in more than one functional system (e.g., ref. 22). In addition, many neurological disorders, such as Parkinson, Alzheimer, and Huntington diseases and, perhaps, epilepsy, are expressed as a loss or dysfunction in one or more neurotransmitter system. Interactions of neurotransmitters may allow for subtleties in neuronal modulation that are important for understanding these profound neurological deficits.

In the present experiments we show that multiple neurotransmitters are involved in determining the amplitude of individual currents and, therefore, the electrophysiological behavior of human neocortical neurons.

METHODS

Human neocortical tissue used was a small portion of tissue which is normally removed for the treatment of intractable epilepsy. In the majority (71%) of cases, the tissue was obtained from the lateral portions of the anterior temporal lobe in patients in which focal epileptiform activity was localized with chronic depth electrodes to more mesial structures (e.g., hippocampus). This neocortical tissue was not grossly abnormal when examined histologically and did not give rise to abnormal synchronous discharges when maintained in vitro. The extent to which the present results are affected by the patient’s history of epilepsy is not known.

Neocortical tissue was resected en bloc by the neurosurgeon (Dennis Spencer, Yale University School of Medicine), and a small portion was placed in 5°C bathing medium. In addition, some experiments were performed on anterior cingulate, sensorimotor, or temporal cortical tissue obtained from guinea pigs. These animals were deeply anesthetized with sodium pentobarbital (20–30 mg/kg i.p.) and decapitated. Four-hundred-micrometer slices were prepared on a vibratome and maintained at the gas/liquid interface in a recording chamber at 35 ± 1°C (23). The bathing medium was 124 mM NaCl/2.5 mM KCl/2 mM MgSO4/1.25 mM NaH2PO4/26 mM NaHCO3/2 mM CaCl2/10 mM dextrose.

Intracellular recording electrodes (30–50 MΩ) contained 4 M potassium acetate. I-V relationships were obtained in discontinuous single electrode voltage clamp by holding the cells at −45 to −60 mV and applying a 10-sec voltage ramp to between −120 and −140 mV and measuring the current required to do so. Head-stage output was continuously monitored to ensure adequate settling time. Sample frequencies were typically between 4 and 5.5 kHz, and amplifier gain ranged from 0.5–1.0 nA/mV.

Methysergide was a gift from Sandoz and 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) was obtained from Research Biochemicals, Natick, MA. Bis(2-aminophenoxo)ethane-N,N',N''-tetraacetic acid (BAPTA) was obtained from Molecular Probes. Ethylene glycol bis(β-aminoethyl ether)-N,N',N''-tetraacetic acid; 5-HT, 5-hydroxytryptamine or serotonin.

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Abbreviations: IAHP, K+ current that underlies the slow afterhyperpolarization; IM, K+ current that is voltage and time dependent; ACh, acetylcholine; MCh, methacholine; GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine or serotonin.

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RESULTS

Stable intracellular recordings were obtained from 110 human and 82 guinea pig cortical neurons for periods of up to 6 hr each. A representative sample of human cortical cells yielded an average resting membrane potential of $-81 \pm 8$ mV (SD; $n = 22$), action potential amplitude of $102 \pm 12$ mV, and apparent input resistance of $43 \pm 10$ MΩ. The electrophysiological properties of neurons studied here indicated that they were most likely pyramidal in morphology (23, 24).

Reduction of Spike-Frequency Adaptation. Intracellular injection of a suprathreshold depolarizing current pulse resulted in a train of action potentials that showed marked spike-frequency adaptation (Fig. 1, control). Extracellular application of ACh (1–5 mM in micropipette), or the muscarinic agonist acetyl-$\beta$-methylcholine (MCh; 1 mM), resulted in a slow depolarization of the membrane potential and a substantial reduction in spike-frequency adaptation, even after the membrane potential was manually repolarized to the preapplication level (Fig. 1B; $n = 7$, human; $n = 3$, guinea pig). Similarly, application of norepinephrine (500 μM; $n = 7$, human; $n = 15$, guinea pig), histamine (500 μM; $n = 6$, human; $n = 11$, guinea pig) and 5-HT (300–500 μM; $n = 4$, human; $n = 6$, guinea pig) also greatly reduced spike-frequency adaptation (Fig. 1A, C, and D). However, norepinephrine never, and histamine and 5-HT only occasionally, depolarized the baseline membrane potential (data not shown).

Spike-frequency adaptation results, in part, from the activation of two separate $K^+$ currents: IAHP and IaM (25). IAHP is activated by increases in the intracellular concentration of unbound Ca$^{2+}$ and underlies the slow afterhyperpolarization (hence its name) occurring after a train of action potentials (26). IAHP was investigated by injecting a depolarizing current pulse (0.8–1.0 nA; 300 msec), which elicited from 6–15 action potentials in normal medium or after a single Ca$^{2+}$-dependent action potential in medium containing 1 μM tetrodotoxin and 5 mM tetraethylammonium. At the termination of the current pulse, the neuron was voltage-clamped (typically at $-60$ mV), and the amplitude and time course of the after current was examined (Fig. 1E–H). Individual trains of action potentials in normal bathing medium were followed by a slow outward current that possessed an average reversal potential of $-100 \pm 6$ mV ($n = 8$). Decay of the later portions of IAHP was well fit ($r > 0.98$) by a single exponential function with an average time constant of $996 \pm 382$ msec; $n = 5$ (26).

Separate applications of MCh, norepinephrine, histamine, and 5-HT all substantially reduced or abolished the slow afterhyperpolarization and/or IAHP ($n = 41$, human; $n = 63$, guinea pig), even in the same neuron (Fig. 1E–H; $n = 2$, human; $n = 5$, guinea pig). This convergence onto IAHP occurred through four distinct postsynaptic receptors. Block of three of the four receptor subtypes involved in guinea pig neurons by adding the muscarinic antagonist scopolamine (1 μM; $n = 9$), the β-adrenergic antagonist propranolol (20 μM; $n = 11$), the 5-HT antagonist methysergide (5 μM; $n = 8$) or the H₂ histaminergic antagonist cimetidine (10–20 μM; $n = 11$) to the bathing medium prevented reduction of IAHP by stimulation of all but the unblocked receptor.

Reduction of IaM. IaM also contributes to spike-frequency adaptation (2, 27). The possibility that ACh, histamine, norepinephrine, and 5-HT may suppress IaM was therefore examined. Application of MCh to all neurons and 5-HT to middle-layer cells, after depolarization with intracellular injection of current to near-firing threshold (e.g., $-60$ mV) resulted in a slow depolarization and a decrease in apparent membrane conductance (Fig. 2A and B; $n = 17$, human). The possibility that this slow depolarization represented a block of IaM was examined by applying voltage steps (1-sec duration) between −45 and −60 mV. The voltage step from −45 to −60 mV was associated with the relaxation of an outward current which was well fit ($r > 0.99$) by a single exponential function with an average time constant of $71 \pm 13$ msec; $n = 12$.

![Fig. 1](image-url)
human). Voltage steps between $-75$ and $-90$ mV, out of the range of $I_M$, were not accompanied by activation of this current (data not shown).

Under voltage-clamp conditions, MCh ($n = 21$, human) or 5-HT ($n = 6$, human) caused a large apparent inward current, lasting from 5–15 min, that resulted from a suppression of $I_M$ (Fig. 2 C–I). The marked voltage dependence of this effect is well illustrated in $I$–$V$ plots obtained before and during MCh and 5-HT (Fig. 2 G, H, and I). Under these conditions, MCh and 5-HT both induced a marked inward shift of the $I$–$V$ relation at membrane potentials positive to approximately $−70$ mV (Fig. 2 G, H, and I). In some neurons, there also appeared to be a smaller, relatively linear inward component of the MCh and 5-HT responses. This component reversed at $−108 ± 4$ mV ($n = 3$, human).

Subtracting the current traces associated with the voltage steps between $−45$ and $−60$ mV before and after MCh or 5-HT revealed three components that can be explained as suppression of $I_M$ (Fig. 2 E and F, differences a–c). The reduction in instantaneous “leak” current associated with the voltage jump from $−45$ to $−60$ mV represents a reduction in the amount of $I_M$ active at $−45$ mV (Fig. 2 E and F, difference a). The slow apparent inward current seen after stepping from $−45$ to $−60$ mV represents the deactivation or “turning off” of that portion of $I_M$ suppressed by MCh and 5-HT (Fig. 2 E and F, difference b), whereas the slow outward

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**FIG. 2.** Activation of muscarinic and 5-HT receptors reduces M-current. Application of MCh (A) or 5-HT (B) to a human cortical neuron depolarized with intracellular injection of current to $−60$ mV results in a slow depolarization and action potential discharge. Compensation for the slow depolarization by adjusting the injected current (top trace) reveals a substantial decrease in apparent input conductance (dashed line). Application of MCh (C and E) or 5-HT (D and F) during voltage steps from $−45$ to $−60$ mV in voltage clamp results in a marked suppression of an outward current, which is turned off by the step to $−60$ mV and activated by the step to $−45$ mV. Current and voltage steps in C and D are expanded for detail in E and F (nos. 1 and 2). Each trace in E and F is an average of between 5 and 16 individual steps, as indicated in C and D. The difference of the currents required to perform the voltage steps before and after MCh and 5-HT reveal three parts labeled a, b, and c and correspond to a decrease in fast “leak” conductance (difference a), and the turning off (difference b) and on (difference c) of $I_M$, (G and H) $I$–$V$ plots before and after application of MCh and 5-HT. Outward rectification in G control is smaller than in H control due to incomplete washout of an application of MCh before G. (I) plot of the difference between the $I$–$V$ curves in G and H versus membrane potential reveals the voltage dependence of the current suppressed by MCh and 5-HT. All data was obtained from the same lower-layer III neuron in the human anterior temporal lobe.
current seen when stepping from -60 to -45 mV represents the activation of that same portion of $I_M$ (Fig. 2 E and F, labeled difference c).

The suppression of $I_M$ by 5-HT is not mediated by muscarinic receptors because local application of scopolamine (10–20 μM, $n = 3$, human) completely blocked the response to MCh but did not alter that to 5-HT. This effect is not due to the suppression of a Ca$^{2+}$ or Na$^+$-activated potassium current (28) because it persisted in neurons recorded for more than an hour with electrodes containing high concentrations of the Ca$^{2+}$-chelating substances BAPTA (0.2 M) or EGTA (0.5 M) ($n = 3$, human) as well as after application of tetrodotoxin (10 μM; $n = 3$, human). In addition, application of norepinephrine, which potently blocks $I_{AHP}$, did not result in any reduction of $I_M$ ($n = 6$, human).

Increase in Membrane Potassium Conductance. Application of adenosine (2 mM in micropipette; $n = 10$, human; $n = 11$, guinea pig), the GABAB receptor agonist baclofen (100 μM; $n = 17$, human; $n = 8$, guinea pig), and in some neurons, 5-HT ($n = 14/30$, human; $n = 13/21$, guinea pig) resulted in a hyperpolarization or an outward current and an increase in membrane conductance (Fig. 3). The average reversal potential for this response, determined from $I-V$ relationships (Fig. 3A), was similar for all three agents (adenosine: $-106 \pm 6$ mV, $n = 6$; baclofen: $-104 \pm 7$ mV, $n = 14$; 5-HT: $-101 \pm 4$ mV, $n = 8$).

In neurons of the rodent hippocampus and brainstem maximal activation of an outward K$^+$ current by baclofen dramatically reduces the outward current induced by 5-HT, and vice versa (20, 21). Similarly, we found that baclofen reversibly reduced the adenosine-induced current by 85 ± 18%; ($n = 4$, human; $n = 4$, guinea pig) and the 5-HT-induced current by 95 ± 9% ($n = 3$, human; Fig. 3 B1 and B2). This nonadditivity suggests convergence of these three agents onto the same potassium current (12, 17).

Local application of the GABAB antagonist phaclofen (5 mM in micropipette) resulted in a substantial (60%) decrease in the response to baclofen, but not adenosine ($n = 4$). Local application of the 5-HT$_{1A}$ partial agonist 8-hydroxydipropylaminotetralin (500 μM), on the other hand, resulted in a small hyperpolarization and increase in membrane conductance ($n = 3$, human; $n = 3$, guinea pig). In addition, local application of 8-hydroxydipropylaminotetralin also resulted in a reduction, or block, of the hyperpolarizing response to 5-HT and the appearance, or enhancement, of a 5-HT-induced slow depolarization (data not shown; $n = 2$, human; $n = 2$, guinea pig). These results indicate that baclofen is causing an increase in membrane potassium conductance through GABAB receptors, whereas 5-HT is doing so through the 5-HT$_{1A}$ receptor subtype.

Anatomic Distribution of Neurotransmitter Responses. The ionic responses to ACh, adenosine, baclofen, histamine, and norepinephrine detailed above appeared to be present in all, or nearly all, presumed pyramidal neurons that we tested in layers II–III and V of the anterior temporal, occipital, and frontal human cortical regions. However, this is not to say that significant laminar and area differences do not exist (for example, see ref. 29). Indeed, the responses to 5-HT, although consistent between applications in the same neuron, varied significantly between different cells. The 5-HT-induced hyperpolarization and outward current was prominent in layer II and V neurons, whereas the suppression of $I_M$ appeared to be more prominent in neurons located in mid-cortical lamina. 5-HT-induced suppression of $I_{AHP}$ was present in all neurons tested, regardless of laminar position. Further experiments on possible laminar and area differences in transmitter actions would be worthwhile.

**DISCUSSION**

Anatomical and psychopharmacological data suggests that the excitability of neurons in human cerebral cortex is under the control of cholinergic, GABAergic, histaminergic, noradrenergic, purinergic, and serotonergic neurotransmitter systems (1–8). Our data reveal that the neurotransmitters and/or neuromodulators thought to be released by these systems have potent and long-lasting effects on specific ionic currents in human cortical pyramidal cells. ACh, histamine, norepinephrine, and 5-HT are potent blockers of spike-frequency adaptation, an effect that results, in part, from a reduction of two specialized K$^+$ currents: $I_{AHP}$ (blocked by all four agents) and $I_M$ (blocked by ACh and 5-HT) (Figs. 1, 2, 3).
release of ACh, norepinephrine, 5-HT, and/or histamine may also lead to an increase in baseline firing rate, although this excitation will be much less than the enhancement of phasic barrages of EPSPs because of the marked activation voltage dependence of IAHP and IM. Furthermore, reduction of these two currents will have much less effect on inhibitory postsynaptic potentials or unitary EPSPs because neither of these results in substantial activation of IAHP or IM.

Convergence and divergence of transmitter actions in the human cerebral cortex complicates our understanding of the control and modulation of neuronal activity. It is likely that in the natural state cortical pyramidal cells are under the constant influence of a dynamically changing array of neuroactive substances. Additive and nonadditive interactions among these substances may allow for subtleties in neuro-modulation that could not otherwise occur. Understanding these actions and interactions may facilitate the development of more specific pharmacological therapies for neurological disorders, such as epilepsy, Alzheimer disease, and age-related cognitive decline.

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