(*19*). Between-strain differences in striatal dopamine receptor number in F344 and BUF rats (*5*) and 11 inbred mouse strains (*20*) are also smaller than the present α-receptor differences. The parallelism in between-strain differences for tritiated agonist and antagonist binding to α_2-receptors indicates a true difference in the density of this receptor in the hypothalamus and medulla-pons; a similar parallelism was observed for dopamine receptor interstrain differences (*20*).

The different PNMT activity in F344 and BUF rat brains may reflect differences in the number of brainstem adrenergic neurons, as previously suggested for SH and WKY rats (*21*). In analyzing genetic differences in brain dopamine systems in two inbred mouse strains, Baker *et al.* (*22*) found quantitatively similar differences in midbrain TH activity and dopamine neuron number.

The present results, in addition to allowing a further genetic analysis of the interrelation between PNMT and α-receptors, suggest a model for examining the regulation of α-receptor number and function in pathological and therapeutic conditions in which both inherited factors and α-adrenergic receptor function are thought to be important (*18, 23*).

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9. Catecholamines were isolated from tissue by alumina adsorption and chromatographed in a 25-cm reverse-phase C-18 column (5 μm; Bioanalytical Systems) with a mobile phase of phosphate buffer, sodium octyl sulfate, and methanol. The catecholamines were detected amperometrically by a Bioanalytical Systems LC-3 electrode system. Dihydroxybenzylamine was added prior to alumina extraction as an internal standard.
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4 January 1983; revised 11 March 1983

Acetylcholine Mediates a Slow Synaptic Potential in Hippocampal Pyramidal Cells

Abstract. *The hippocampal slice preparation was used to study the role of acetylcholine as a synaptic transmitter. Bath-applied acetylcholine had three actions on pyramidal cells: (i) depolarization associated with increased input resistance, (ii) blockade of calcium-activated potassium responses, and (iii) blockade of accommodation of cell discharge. All these actions were reversed by the muscarinic antagonist atropine. Stimulation of sites in the slice known to contain cholinergic fibers mimicked all the actions. Furthermore, these evoked synaptic responses were enhanced by the cholinesterase inhibitor eserine and were blocked by atropine. These findings provide electrophysiological support for the role of acetylcholine as a synaptic transmitter in the brain and demonstrate that nonclassical synaptic responses involving the blockade of membrane conductances exist in the brain.*

The first identification of a synaptic transmitter in the vertebrate central nervous system (CNS) was in 1954 when Eccles *et al.* (*1*) demonstrated that the collaterals of spinal motoneurons use the transmitter acetylcholine (ACh) to excite Renshaw cells. Although a variety of studies since then have suggested that ACh might be a transmitter at various sites in the mammalian CNS (*2–6*), no convincing correlation at the intracellular level has been made between the actions of ACh and synaptic responses (*7–9*). We examined this question in the hippocampal slice preparation, in which pyramidal cells receive a dense cholinergic input from the medial septum (*7, 10*). Using intracellular recording in this preparation, we found that the muscarinic actions of ACh, which are slow and involve nonclassical ionic mechanisms (*3–6*), can be mimicked exactly by synaptic inputs. These inputs are blocked by the muscarinic antagonist atropine and are enhanced by the cholinesterase inhibitor eserine.

We have described the details of the rat hippocampal slice preparation elsewhere (*11*). Intracellular recordings were obtained from over 100 CA1 pyramidal cells using 2*M* potassium methyl sulfate–filled microelectrodes. Drugs were applied by superfusion. Stimulating electrodes were positioned in the CA2/CA3 region of stratum oriens to activate the cholinergic fibers that are concentrated there (*10*). A 20- to 40-Hz train of stimuli for 0.5 seconds was optimal. Stimulus

intensity was usually adjusted to evoke a threshold cholinergic response. Eserine (1 to 10 μM) was then added to the perfusate and the stimulation repeated. Finally, atropine (50 nM to 1 μM) was added to the perfusate and the stimulation repeated again. Such a paradigm permitted us to demonstrate the effects of eserine and atropine on the responses in the same cell. However, all of the responses to be reported were also clearly seen in the absence of eserine and were blocked by atropine.

In concentrations above 20 μM, ACh had three effects on pyramidal cells (Fig. 1). First, it depolarized the membrane; this was associated with an increase in neuronal input resistance as measured by constant-current hyperpolarizing pulses (downward deflections in Fig. 1B) (*3–6*). The increase in resistance was most clearly seen when the membrane potential was hyperpolarized back to the resting level with d-c current (see bar beneath record in Fig. 1B). This action of ACh is thought to be largely due to blockade of the M-current (*6*). Second, ACh blocked the slow afterhyperpolarization (AHP) that follows a short train of action potentials evoked by a depolarizing current pulse (compare trace 1 in Fig. 1A to trace 1 in Fig. 1C) (*5, 12*). This AHP is due largely to a calcium-activated potassium conductance (*13*). Third, the accommodation of cell discharge that occurs with long-duration current pulses was severely reduced (compare trace 2 in Fig. 1A to trace 2 in Fig. 1C). This effect was not simply due to the larger depolarizing response obtained in the presence of ACh, because after the addition of atropine the cell still accommodated even when the depolarization was increased to match that obtained in the presence of ACh (compare trace 2 in Fig. 1E to trace 3 in Fig. 1E). Both M-current blockade (*6, 14*) and blockade of calcium-activated potassium conductance (*15*) would be expected to attenuate accommodation. Each of the effects of exogenously applied ACh was effectively reversed by the muscarinic antagonist atropine (Fig. 1, D and E) and unaffected by the nicotinic antagonist dihydro-β-erythroidine (40 μM). The same spectrum of effects was seen with a number of cholinomimetics, including muscarine, pilocarpine, and carbachol. Nicotine had no effect on pyramidal cells.

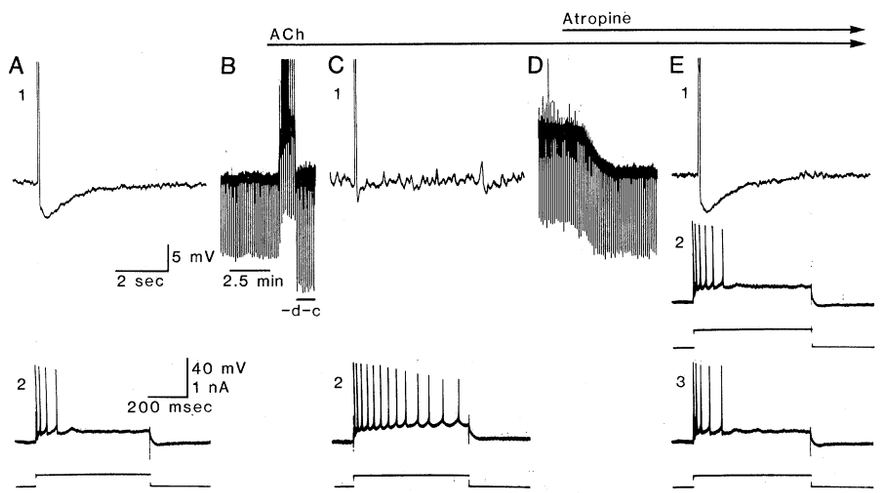

Fig. 1. Effects of ACh on hippocampal pyramidal cells. All responses are from the same CA1 pyramidal cell. (A) Chart record of control AHP after a 60-msec direct depolarizing current pulse (trace 1) and film record of response to a 600-msec pulse (trace 2). The current record is positioned below the voltage record. (B) ACh (200 μM) superfusion depolarized the membrane and increased the cell's input resistance. (C) Blockade of the AHP (trace 1) and accommodation (trace 2) in the presence of ACh. (D) The addition of atropine (0.5 μM) in the presence of ACh reversed the effects of ACh. (E) Atropine also reversed the effect of ACh on the AHP (trace 1) and on accommodation (traces 2 and 3). The current pulse in trace 3 was identical to those in trace 2 in (A) and (C). In trace 2 the current pulse was increased to match the depolarization evoked in the presence of ACh [trace 2 in (C)]. The gain in trace 1 in (A) applies to all of the chart records. The time calibration for trace 1 in (A) also applies to trace 1 in (C) and (E), and the time calibration in (D) is the same as that in (B). The calibration for trace 2 in (A) applies to all the film records. Resting membrane potential, −57 mV.

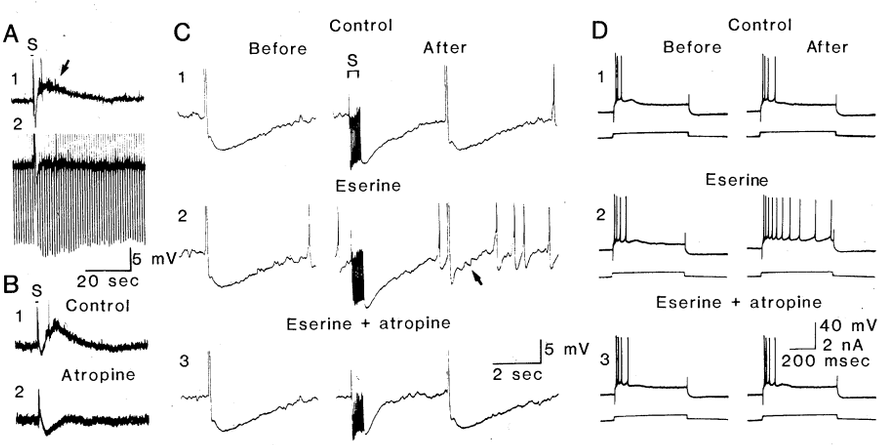

Fig. 2. Effect of exogenous ACh mimicked by stimulating the stratum oriens. Records from four different cells are shown in (A) to (D). (A) Immediately after a train of stimuli (*S*), the cell hyperpolarizes (condensed on this time scale) and then depolarizes (arrow in trace 1). In trace 2 the slow depolarization was manually voltage-clamped which clearly shows the increase in size of the hyperpolarizing current pulses. Resting membrane potential, −53 mV. In another cell (B), slow depolarization (trace 1) was completely blocked by 1 μM atropine (trace 2). Resting membrane potential, −59 mV. [Calibration in (A) also applies to (B).] (C) AHP's before pathway stimulation and 4 seconds after the stimulus. In control solution (trace 1) the AHP was slightly depressed after the stimulus; after the addition of 1 μM eserine (trace 2) the AHP was greatly reduced by the stimulus (arrow); and the AHP was restored to control amplitude by 1 μM atropine (trace 3). Resting membrane potential, −56 mV. (D) Identical experimental protocol as in (C), except showing the accommodation during a 600-msec pulse before and after pathway stimulation. Resting membrane potential, −64 mV.

We sought to duplicate these actions of ACh by stimulating sites in the slice known to contain cholinergic fibers (*10*). While responses could be evoked from a number of stimulating sites, in general the strongest effects were obtained by stimulating stratum oriens in the CA2/CA3 region. Stimulation in this region (S in trace 1 of Fig. 2A) produced a noncholinergic hyperpolarization lasting 2 to 3 seconds, followed by a slow cholinergic depolarization lasting 20 to 30 seconds (arrow in trace 1 of Fig. 2A). This depolarization was associated with an increase in neuronal input resistance (trace 2 in Fig. 2A). The size of the voltage deflections to hyperpolarizing current pulses was increased when the slow depolarization was manually voltage-clamped. The addition of eserine increased the size and duration of this depolarization, resulting in spike dis-

charge. Atropine blocked the depolarization (Fig. 2B). These findings indicate that this potential is a slow muscarinic excitatory postsynaptic potential (EPSP).

In addition to generating a slow EPSP, the stimulus also reduced the AHP after activation of the cell by depolarizing current pulses, indicating a reduction in calcium-activated potassium conductance. In Fig. 2C an AHP was elicited before and 4 seconds after pathway stimulation (S) that caused a small decrease in the amplitude of the AHP. In the presence of eserine (trace 2 in Fig. 2C) the same stimulus caused a much larger reduction (arrow). Atropine entirely blocked the stimulus-induced depression of the AHP (trace 3 in Fig. 2C). Pathway stimulation also attenuated accommodation of spike discharge during long direct depolarizing current pulses, and this effect was markedly enhanced by eserine and blocked by atropine (Fig. 2D). Both the AHP and the accommodation provided very sensitive measures of cholinergic activity, since dramatic effects on these parameters could be seen in the absence of any change in membrane potential (*16*).

These results demonstrate that electrical stimulation of sites known to contain cholinergic fibers can evoke synaptic responses in hippocampal pyramidal cells that exactly mimic all the postsynaptic effects of exogenously applied acetylcholine. Thus the findings fulfill an important criterion in establishing ACh as a neurotransmitter and indicate that the pharmacological effects produced by ACh are indeed of physiological relevance. Except for two reports (*8, 17*), chemical synapses in the mammalian brain have been found to increase membrane conductance. The cholinergic slow EPSP reported here is nonclassical in that at least two types of potassium conductances (calcium-activated and M-current) are blocked; both exert powerful braking actions over repetitive cell discharge (*2, 14, 15*). As a consequence, during cholinergic synaptic activity, pyramidal cells fire many more action potentials to depolarizing stimuli, even though the synaptic action may cause little or no depolarization of the cell. Norepinephrine (*15*) and serotonin (*18*) have also been found to facilitate repetitive discharge.

Early iontophoretic studies indicated that ACh could decrease resting potassium conductance in neocortical neurons (*3*), and a number of recent studies, primarily of invertebrate neurons and neurons from the vertebrate peripheral nervous system, have emphasized the importance of voltage- and calcium-dependent potassium conductances as targets for neurotransmitter action (*19*). The present results indicate that such nonclassical actions also occur in neurons of the mammalian brain following the synaptic release of ACh.

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17 February 1983; revised 23 May 1983

Stress-Induced Suppression of Immunity in Adrenalectomized Rats

Abstract. *Stress-induced suppression of lymphocyte stimulation by phytohemagglutinin was demonstrated in isolated lymphocytes and in cultures of whole blood from adrenalectomized rats. The results demonstrate that corticosteroid independent mechanisms participate in the suppression of lymphocyte function by stressors. Stress-induced lymphopenia, however, was found to be adrenal dependent, indicating that the modulation of immunity by stress is complex and multidetermined.*

We showed previously that a graded series of stressors suppressed lymphocyte function in rats (*1*). Secretion of corticosteroids has long been considered to be the mechanism of stress-induced modulation of immunity and related disease processes (*2–4*). Concurrent increased adrenal activity, suppressed measures of immunity, and increased illness susceptibility have been reported (*5–7*). These studies have suggested that lymphocyte function is suppressed by stress-induced adrenal activity. In the present study we investigated the effects of stressors in adrenalectomized rats to determine if the adrenal is required for stress-induced suppression of lymphocyte function.

We used a total of 160 W/Fu rats (Microbiological Associates) and a four-times-four experimental design (ten rats per group). The four groups of rats studied consisted of nonoperated, andrenalectomized, sham adrenalectomized, and adrenalectomized animals with a corticosterone pellet. The four treatments—home-cage control, apparatus control, and low-shock and high-shock animals—