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Acetylcholine Modifies Neuronal Acoustic Rate-Level Functions in Guinea Pig Auditory Cortex by an Action at Muscarinic Receptors

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ABSTRACT Cholinergic modification of neuronal responsiveness in auditory cortex includes alteration of spontaneous and tone-evoked neuronal discharge. Previously it was suggested that the effects of acetylcholine (ACh) and muscarinic agonists on neuronal discharge resembled those due to increases in the intensity of acoustic stimuli (Ashe et al. 1989). To determine the relationship between neuronal modifications due to ACh acting at muscarinic receptors and those due to changes in stimulus intensity, we determined acoustic rate-level functions for neurons in the auditory cortex of barbiturate-anesthetized guinea pigs before, during and after administration of ACh. ACh facilitated acoustic rate-level functions in 82% of the cells tested. In addition, during ACh administration 66% of neurons responded to stimuli that were previously subthreshold, that is, ACh decreased the response threshold. Cholinergic facilitation of rate-level functions was attenuated by the general muscarinic antagonist atropine. The nature of the muscarinic receptors involved in the actions of ACh was further examined by presenting single tones before, during, and after administraton of ACh and specific muscarinic receptor subtype antagonists, either pirenzepine (M_1) or gallamine (M_2). ACh-induced facilitation of spontaneous and tone evoked neuronal discharge was antagonized by pirenzepine, but not by gallamine, suggesting the involvement of the M_1 muscarinic receptor subtype. These data indicate that ACh can facilitate stimulus-evoked responses and decrease response thresholds for neurons in auditory cortex, possibly via activation of M_1 muscarinic receptors. Such effects of ACh acting at muscarinic receptors could underly cholinergic regulation of information processing in the auditory cortex.

Acetylcholine (ACh) modifies spontaneous and evoked discharge of neurons in sensory koniocortex. Often the effects of ACh result in systematic modification of the receptive field of the neuron (for recent reviews, see Ashe and Weinberger, 1990; Weinberger et al., 1990). In auditory neocortex, modifications of the neuronal frequency receptive field consist of a change in frequency selectivity and/or a shift in the best frequency that result from simultaneous facilitation and reduction of responses to different frequencies of acoustic stimuli (Ashe et al., 1989; McKenna et al., 1989; Metherate and Weinberger, 1989, 1990). Modification of evoked discharge rate and acoustic receptive field are the result of ACh acting at muscarinic receptors (Ashe et al., 1989; McKenna et al., 1988; McKenna et al., 1989). Moreover, the change in frequency receptive field that is produced by ACh also can be produced by classical conditioning procedures (Weinberger and Diamond, 1987). These data, along with the known involvement of muscarinic actions in the control of neuronal excitability (Brown, 1988; Nicoll, 1988), have led to the hypothesis that the activation of muscarinic receptors in auditory cortex can produce alterations of the neuronal response that are similar to those resulting from an increase in the intensity of acoustic stimuli (Ashe et al., 1989). Here we report the initial results of a test of this hypothesis. Furthermore, we also report on the differential involvement of subtypes of the muscarinic receptor in mediating the effects of ACh in auditory cortex.

Methods of recording single unit discharge, acoustic stimulation, and iontophoretic application of pharmacological agents used were similar to those previously reported (McKenna et al., 1988; Metherate and Weinberger, 1989). Briefly, discharges were recorded from neurons (n = 34) located in the rostral tonotopic field of adult guinea pigs anesthetized with sodium pentobarbital (Nembutal, 35 mg/kg, i.p.), and supplemented with

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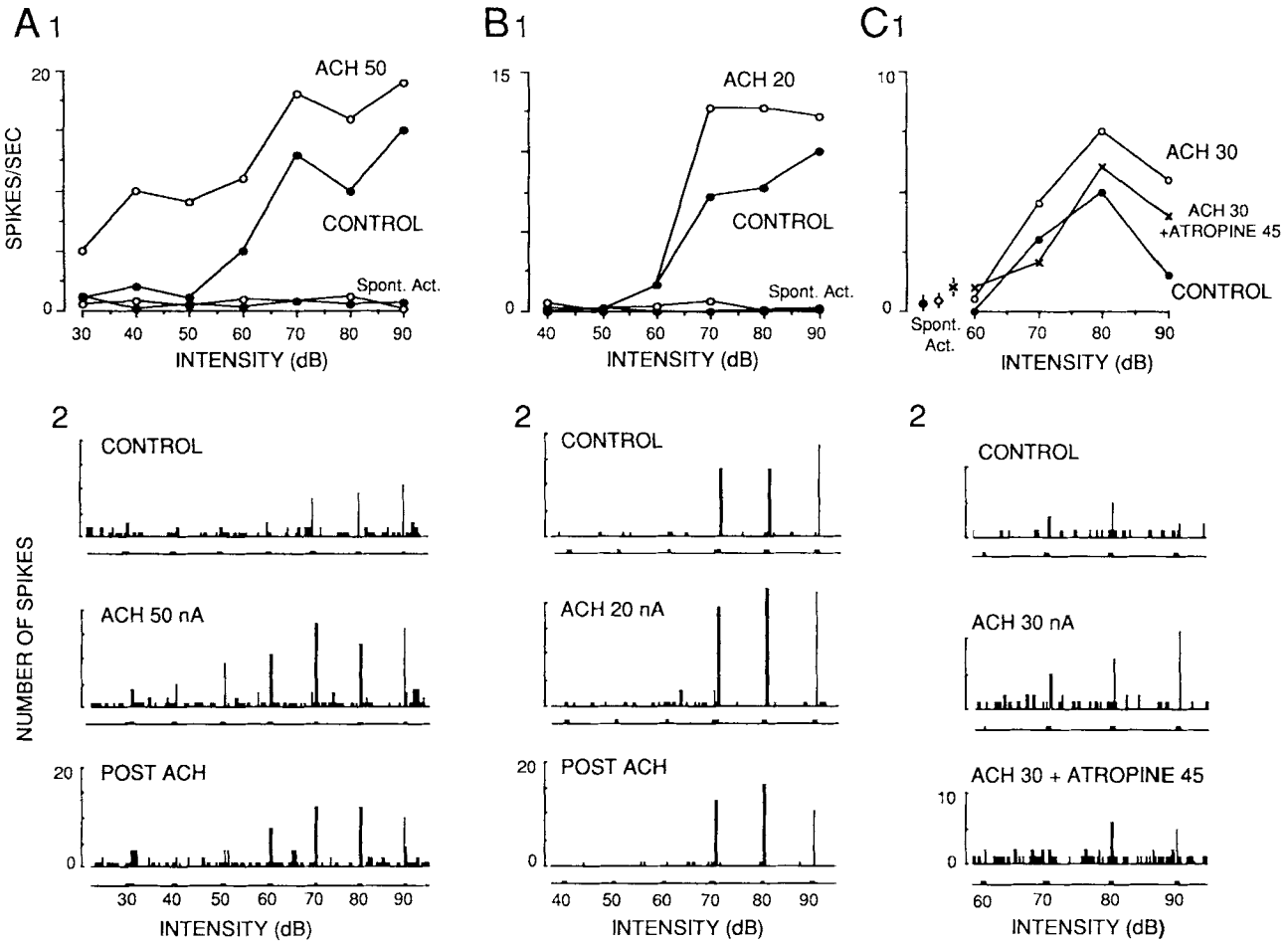

Fig. 1. ACh-induced modification of rate-level functions for 3 cells (A, B, and C). For each cell, rate-level functions and mean levels of spontaneous activity are shown at the top of the figure, while the histograms below indicate the pattern of neural discharge during presentation of acoustic stimuli (bar). Acoustic stimulation consisted of 20 repetitions of an ascending intensity sequence of tones (in 10 dB steps) presented at the contralateral ear. A: This cell originally discharged above spontaneous levels in response to stimuli of 60–90 dB intensities, resulting in a monotonically increasing rate-level function (Control). ACh (50 nA) facilitated the magnitude of the evoked responses without changing the rate of spontaneous discharge. Additionally, in the presence of ACh, responses were clearly elicited by stimulus intensities as low as 30 dB, i.e., ACh reduced the response threshold. The effects of ACh on response magnitude and threshold were partially dissipated 3 minutes after ACh (Post-ACh histogram). B: In another cell, ACh (20 nA) facilitated the previously evoked responses (70–90 dB) but did not alter either the response threshold or the level of spontaneous activity. C: This cell originally responded only to 70 and 80 dB stimuli, whereas higher and lower intensity stimuli were relatively ineffective. The resulting rate-level function increased nonmonotonically with intensity (Control). ACh (30 nA) facilitated the responses to 70–90 dB stimuli but did not alter either the discharge to lower intensity stimuli or the level of spontaneous activity (for clarity, the level of spontaneous activity (mean ± SD) for each condition is shown to the left of the rate-level functions). Atropine (45 nA) partially antagonized the ACh-induced facilitation of the evoked response, suggesting the involvement of muscarinic receptors in the action of ACh. Tone duration 50 ms, histogram bin width 10 ms for all cells.

an analgesic/tranquilizer (Innovar-Vet, 4 mg/kg). The use of barbiturate anesthesia would be expected to reduce neuronal excitability (Nicoll and Madison, 1982), but has proved useful in parametric studies of cholinergic function in sensory cortex as it also results in reduced response variability (Metherate et al., 1988; Metherate and Weinberger, 1989). The acoustic stimulation system was calibrated to produce tones of specified intensity (SPL re: 20 μPa) at the contralateral ear. Neuronal activity was led to a laboratory computer for generation of on-line histograms, and rate meter output was monitored with a chart recorder. Cholinergic modulation of discharge rate in response to increasing sound pressure levels was investigated by determining rate-level functions (at "best" frequency) before, during, and after iontophoretic administration of ACh. The best frequency was considered the frequency that elicited the largest number of discharges at a level 20–30 dB above threshold.

The predominate effect of ACh was to increase the magnitude of acoustically evoked discharge resulting in a facilitation of rate-level functions (9/11 neurons) (Fig. 1; Table IA). The rate of spontaneous discharge could also be increased by ACh (6/11 cells, Table IA). The effects of ACh on the discharge rate of auditory cortical neurons has been shown in several studies to be mimicked by muscarinic agonists (McKenna et al., 1988, 1989), and blocked by the muscarinic antagonist atropine (Ashe et al., 1989; Metherate and Weinberger, 1989). Similarly, atropine (50 mM solution in pipette) was effective in antagonizing ACh-induced facilitation of rate-level functions (n = 2; Fig. 1C). The effect of ACh

TABLE I. Effects of ACh and muscarinic antagonists on auditory cortical neurons

A. ACh-induced modification of response threshold and cell discharge (n = 11)

	No. of cells	% of Total
I. Response threshold		
Decrease	6	54
Increase	1	9
No change	4	36
II. Cell discharge[1]		
a. Evoked		
Facilitation	9	82
Reduction	2	18
No change	0	0
b. Spontaneous		
Facilitation	6	54
Reduction	0	0
No change	5	45

B. Effects of muscarinic antagonists on ACh-induced modification of cell discharge (n = 14)

	Antagonism			
	Pirenzepine		Gallamine	
ACh effect[2]	Frequency	%	Frequency	%
Facilitation	7/10	70	2/7	28
Reduction	1/1	100	3/3	100

[1]Evoked discharge is the average rate during the 50 msec tone duration; spontaneous rate is the average discharge during the 500 msec period that preceded each tone. In 10/11 cells, the changes in evoked and spontaneous activity either co-varied (5 cells), or the evoked discharge was modified while spontaneous activity was not (5 cells). In one cell ACh both facilitated spontaneous activity and reduced evoked discharge.
[2]Includes effects on evoked and/or spontaneous activity. Changes in evoked and spontaneous activity did not vary inversely; that is, when both changed, the change was in the same direction or one changed while the other did not. For 7 cells that did not respond to tones, spontaneous activity was measured using the ratemeter output.

was not necessarily limited to the responsive range of the rate-level function. That is, in addition to facilitation, exposure to ACh resulted in a shift of the function to lower stimulus levels. Thus, stimuli that previously were subthreshold for eliciting discharge above the spontaneous rate were now effective during ACh administration (Fig. 1A, Table IA). The decrease in the minimum stimulus intensity necessary to elicit a response ranged from 10 dB (n = 2) to greater than 20 dB (n = 4) during ACh administration (Fig. 1A).

Although ACh facilitation was accompanied by a shift of rate-level functions to lower intensities in 6 of 9 cells (66%) this may be a conservative estimate of the ability of ACh to decrease response thresholds. This is because measurement of the rate-level function was obtained with 10 dB resolution. However, it is possible that higher resolution would reveal a greater frequency of ACh reduction of response threshold, although the reduction may be of lesser magnitude. For the remaining 3 cells in which ACh application resulted in facilitation of the rate-level function, modification of the response was not accompanied by a change in threshold (Fig. 1B, C). Modification of rate-level functions by ACh could occur regardless of whether the cell's rate-level function was monotonic (n = 7; Fig. 1A, B) or nonmonotonic (n = 4; Fig. 1C). However, in no instance did ACh change a neuron's rate-level function from monotonic to nonmonotonic or vice versa.

Within any particular neuron, ACh resulted in either facilitation *or* reduction of the individual responses to the tones that made up the rate-level functions (10/11, 91% of the cells); a mix of facilitation *and* reduction of discharge rate of responses within the rate-level function occurred for only one cell. Thus, interestingly, the qualitatively similar effect of ACh on responses throughout an isofrequency rate-level function stands in contrast to cholinergic modulation of isointensity frequency functions in unanesthetized animals, where ACh often reduces responses to some frequencies while facilitating responses to others (Ashe et al., 1989; McKenna et al., 1989). Therefore, it appears that cholinergic mechanisms are involved in the modification of auditory information processing by an action that can be revealed by examining the stimulus dimensions of intensity and frequency. Whether ACh influences the processing of both intensity and frequency information by the same cellular mechanism(s) remains to be determined. Nor is it known whether identical cellular mechanisms govern ACh induced facilitation and reduction of spike discharge. However, it is known that these mechanisms involve activation of muscarinic receptors, i.e., both the rate of discharge and the sensitivity of the cells to stimuli can be modified by muscarinic agonists or antagonists (Fig. 1C; Ashe et al., 1989; McKenna et al., 1989). Likewise, activation of muscarinic receptors can result in either an increase or decrease in the rate of cellular discharge (Ashe et al., 1989; McKenna et al., 1988; McKenna et al., 1989).

Molecular biological and pharmacological criteria have provided evidence for muscarinic receptor subtypes in the nervous system (for recent review, see Levine and Birdsall, 1989). However, the most extensively studied with regard to synaptically mediated responses are those muscarinic receptors that are generally termed M_1 and M_2 (for review, see Ashe and Weinberger, 1990). The rapid accumulation of knowledge regarding the M_1 and M_2 muscarinic subtypes has occurred largely because of the availability of antagonists that are relatively specific and selective for either M_1 or M_2 binding sites, and also selectively block synaptic responses generated by ACh actions at muscarinic receptors (for review, see Ashe and Weinberger, 1990). Pirenzepine is a specific antagonist at M_1 muscarinic receptors (Hammer and Giachetti, 1982) whereas gallamine is a specific antagonist at M_2 muscarinic receptors, having virtually no effect on neuronal nicotinic cholinergic receptors at concentrations that block muscarinic synaptic responses (Ashe and Yarosh, 1984; Yarosh et al., 1990).

Muscarinic antagonists were used to examine the nature of the muscarinic receptors that mediate the effects of ACh on spontaneous and evoked discharge. A total of 14 neurons were studied; seven of these responded with a pronounced increase in rate when presented with appropriate acoustic stimuli, and seven were unresponsive to stimuli over the range tested. For cells that were responsive to acoustic stimuli, the best frequency tone at suprathreshold intensity was used. These parameters were initially determined by examination of single unit frequency and rate-level functions. Following the assessment of the nature of the change in tone-evoked and/or spontaneous discharge rate, pharmacological agents were administered along with ACh. Pirenzepine (pirenzepine dihydrochloride, 50 mM) was used to assess the involvement of M_1 receptors, and gallamine (gallamine triethiodide, 50 mM) was used to test for M_2 receptors.

The major effect of pirenzepine was to antagonize the

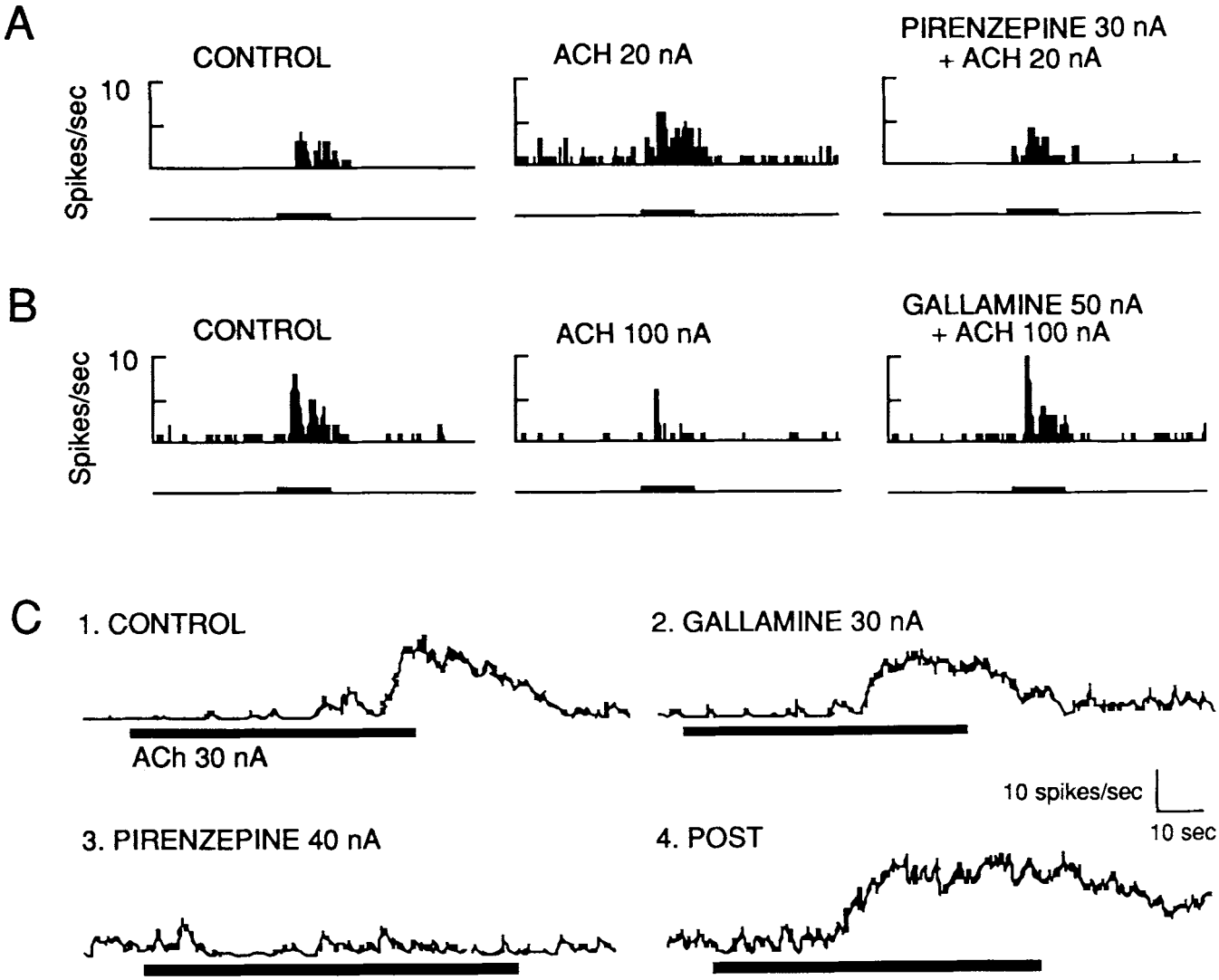

Fig. 2. Effect of selective muscarinic receptor antagonists on ACh-induced modification of cell discharge. A: This cell originally discharged only during presentation of the best frequency acoustic stimulus [frequency 16 kHz, intensity 60 dB, duration 200 ms (bar)]. ACh (20 nA) facilitated the magnitude of the evoked response and produced spontaneous activity. Pirenzepine (30 nA administration begun 2 minutes prior to ACh) effectively antagonized both of these actions of ACh. B: In this cell, ACh (100 nA) strongly reduced the magnitude of the evoked response (24 kHz, 80 dB tone) as well as the rate of spontaneous activity. Gallamine (50 nA administration begun 2 minutes prior to ACh) blocked the ACh-induced reduction of evoked and spontaneous discharge. C: This cell did not respond to acoustic stimuli, and as a result was tested for modification of spontaneous rate only. The rate meter record depicts ACh-induced facilitation of cell discharge (top left). Gallamine (30 nA) did not attenuate the effect of ACh, but pirenzepine (40 nA) did reduce discharge, suggesting that activation of M_1 muscarinic receptors contributed to the actions of ACh. The ACh effect recovered several minutes after termination of the antagonist application. Histogram bin width 5 ms in A and B.

facilitatory effect of ACh on spontaneous and tone-evoked activity (Fig. 2A; Table IB). The antagonistic effect of pirenzepine was highly consistent, resulting in blockade of the facilitation of cell discharge in 7 of 10 cells. In contrast to the effect of pirenzepine, gallamine was relatively ineffective for blockade of ACh-induced facilitation of cell discharge (Fig. 2C; Table IB). Gallamine effectively blocked the ACh-induced reduction of discharge rate in 3 of 3 cells (Fig. 2B). An important demonstration of antagonist selectivity would be differential antagonism of ACh effects by pirenzepine and gallamine when applied to the same neuron. This was tested on 4 cells where ACh produced an increase in discharge rate. For each of these cells, ACh-induced facilitation was effectively blocked by pirenzepine, whereas gallamine was ineffective in 3 of the 4 neurons (Fig. 2C). While full assessment of the differential and selective effects of specific muscarinic antagonists on these neurons requires additional investigation, including full dose-effect functions, these data do provide initial observations suggesting that the muscarinically mediated increase in cell discharge produced by ACh results from an action at the M_1 muscarinic receptor subtype.

Overall, these data support the hypothesis that one consequence of muscarinic receptor activation in audi-

tory cortex is the alteration of neuronal responses similar to that resulting from an increase in stimulus intensity (Ashe et al., 1989). ACh acting at muscarinic receptors cannot only facilitate the rate of cell discharge, but can also decrease response threshold. These findings also suggest that cholinergic mechanisms may likely be involved in the regulation, or "filtering," of auditory information processing. A function for auditory cortical neurons as "adaptive filters" of auditory information that are modified by experience has been previously proposed (Diamond and Weinberger, 1989). Furthermore, the present study provides evidence which suggests that the muscarinic effects of ACh in auditory cortex are complex and may involve mechanisms gated by the M_1 muscarinic receptor subtype.

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