ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1996, 56: 863-872

Cholinergic modulation of synaptic transmission in horizontal connections of rat motor cortex

Grzegorz Hess and **Roman Krawczyk**

Department of Animal Physiology, Institute of Zoology, Jagiellonian University, 6 Ingarden St., 30-060 Cracow, Poland,
Email: Hess@zuk.iz.uj.edu.pl

Abstract. The influence of compounds interacting with cholinergic systems on field potentials evoked in layer II/III horizontal connections was investigated in rat motor cortex *in vitro*. The cholinesterase inhibitor eserine (10 μM) decreased field responses by 20±2%. This effect could be prevented by preincubation with atropine (10 μM). Application of 5 μM carbachol resulted in reduction of the responses by 30±1%. These reductions were reversible, repeatable and independent of stimulus intensity; they could be blocked by the M1 muscarinic receptor antagonist pirenzepine (3 μM) but not by the M2 muscarinic receptor antagonist gallamine (10 μM). During carbachol application, paired-pulse facilitation (40 ms interpulse interval) was increased. The results indicate that endogenous acetylcholine may modulate excitatory synaptic transmission in horizontal connections of rat motor cortex, most likely by acting upon M1 receptors located presynaptically on glutamatergic terminals, and may contribute both to information processing and synaptic plasticity within the motor cortex.

Key words: cerebral cortex, brain slices, carbachol, muscarinic antagonists, cholinesterase inhibitor

INTRODUCTION

The cerebral cortex of mammals receives widespread cholinergic innervation (e.g. Lysakowski et al. 1989, Mrzljak et al. 1995) thought to be important for cortical activation related to arousal (Metherate et al. 1992, Inglis and Fibiger 1995) and to learning and memory (Hagan and Morris 1989, Hasselmo et al. 1992, Winkler et al. 1995). Further, the degeneration of cholinergic neurons occurring in Alzheimer disease accompanies the loss of cognitive functions (Coyle 1983). Acetylcholine may modulate sensory responses in rat cerebral cortex (Donoghue 1987, Donoghue and Carroll 1987, Lewandowski et al. 1993). Studies using *in vitro* preparations have demonstrated that acetylcholine, acting through muscarinic receptors, may induce a variety of effects on membrane properties of neocortical pyramidal neurons, which lead to increased cell excitability (McCormick and Prince 1986, Andrade 1991, Benardo 1993). The activation of muscarinic receptors also influences the firing pattern of pyramidal cells (Metherate et al. 1992, Wang and McCormick 1993). These effects are accompanied by a decrease of synaptic potentials (Yamamoto and Kawai 1967, McCormick and Prince 1986, Vidal and Changeux 1993, Murakoshi 1994). Cholinergic receptors located on glutamatergic presynaptic terminals have been implicated in this kind of modulation of excitatory synaptic transmission. For example, it has been reported that the reduction of excitatory postsynaptic potentials in the prelimbic cortex is mediated by presynaptic M2 muscarinic receptors (Vidal and Changeux 1993). Presynaptic M1 muscarinic receptors have been implicated in the reduction of responses in the hippocampal CA1 area (Sheridan and Sutor 1990). In the piriform cortex, cholinergic agonists selectively suppress responses resulting from intrinsic fiber activation, by a presynaptic M1 receptor-dependent mechanism, while responses resulting from afferent fiber stimulation are largely unaffected (Hasselmo and Bower 1992). In contrast, in some neocortical pyramidal cells nicotinic agonists may potentiate synaptic responses (Vidal and Changeux 1993). It is not known whether cholinergic systems modulate synaptic transmission in horizontal connections within motor cortex.

It has been reported that cholinergic activation may play a role in the induction of long-lasting changes of synaptic efficacy. Dose-dependent effects of application of the cholinergic agonist carbachol were observed in the hippocampus (Auerbach and Segal 1994, 1996), where submicromolar concentration of carbachol induced a long-lasting increase of synaptic responses, resembling long-term potentiation. In contrast, applications of the drug in higher concentrations resulted in a reversible decrease of responses. It has also been demonstrated that activation of cholinergic receptors may influence the potential for the induction of activity-dependent long-lasting changes of synaptic transmission within the hippocampus (Ito et al. 1988, Williams and Johnston 1988, Hirotsu et al. 1989, Burgard and Sarvey 1990, Maeda et al. 1994) and visual cortex (Bröcher et al. 1992). In the auditory cortex, cholinergic agonists acting on muscarinic receptors produce a lasting enhancement of glutamate-mediated membrane depolarizations (Cox et al. 1994). It has recently been shown that synaptic transmission within layers II/III horizontal connections of rat motor cortex is mediated by non-NMDA and NMDA subtypes of glutamate receptors (Aroniadou and Keller 1993, Hess et al. 1994). These connections are capable of long-term potentiation and long-term depression (Hess and Donoghue 1994, Hess and Donoghue 1996, Hess et al. 1996). To begin to investigate a possible role of the cholinergic system in the modulation of these long-term changes, the influence of cholinergic agonists and antagonists on field potentials evoked in horizontal pathways was tested in the present study.

METHODS

Coronal slices were prepared from young adult Wistar rats of both sexes (150-250 g) as described elsewhere (Hess et al. 1994). In brief, animals were deeply anesthetized with sodium pentobarbital, their brains removed from the skull and immersed

in a cold (5-7 °C) artificial cerebrospinal fluid (ACSF) containing (mM): NaCl (126), KCl (3), NaH_2PO_4 (1.25), $MgSO_4$ (2), $CaCl_2$ (2), $NaHCO_3$ (26) and dextrose (15), bubbled with a mixture of 95% O_2-5% CO_2. Coronal slices (400 µm thick) were cut from a part of the cortex corresponding to the primary motor area (Donoghue and Wise 1982) using a vibrating microtome. Slices were then transferred to a fluid-gas interface chamber perfused with ACSF (34.5±0.5 °C) at a rate of 0.5-1 ml/min. The humidified atmosphere over the slices was saturated with a mixture of 95% O_2-5% CO_2. Concentric bipolar platinum/stainless steel stimulating microelectrodes (FHC, USA) were placed 2-4 mm lateral to the midline, 250-350 µm below the cortical surface (layers II/III). Constant-current (0.2 ms, 20-150 µA) pulses were delivered at 0.033 Hz, with an interval of 15 s between stimulation at either electrode in cases when 2 stimulating electrodes were used. Field potentials were recorded using glass micropipettes filled with 0.25 M NaCl (resistance: 3-5 MΩ) at sites displaced by 500-800 µm from the stimulating electrodes. Data were amplified, filtered (0.1 Hz - 1 kHz), acquired at a 10 kHz sampling rate on a Macintosh Quadra 700 computer-based system (National Instruments, USA, A/D board) and analyzed on- and off-line using Igor software (Wavemetrics, USA). Drugs were applied in the bathing ACSF: eserine (10 µM), atropine sulfate (10 µM), carbamylcholine chloride (carbachol, 0.5-100 µM), pirenzepine (3 µM), gallamine triethiodide (10 µM), all purchased from Sigma. In some experiments carbachol (1 mM) was drop-applied (drop diameter: approx. 50 µm) to the surface of the slice. The results were obtained from 30 slices taken from 26 rats and are presented as means ±SEM unless noted.

RESULTS

To investigate the influence of endogenously released acetylcholine upon responses evoked in layer II/III horizontal pathways, 10 µM eserine, an acetylcholinesterase inhibitor, was bath-applied. As illustrated in Fig. 1A, eserine application lasting 15 min induced initially a small, transient increase followed by decrease of horizontally evoked field potential amplitude to 80±2% of control (n=6). A slow recovery of responses close to pre-drug amplitude was observed within about 60 min of drug washout (Fig. 1A). Continued washout was usually accompanied by a small increase of responses above baseline amplitude (on average 11%, n=6; see Fig. 1A), but in two of 6 cases no marked difference between control responses and those recorded after 110 min of eserine washout was observed (see Fig. 1B). The reduction of field responses by eserine could be prevented by atropine sulfate, an unspecific muscarinic receptor antagonist. Figure 1C shows that after perfusion with 10 µM atropine lasting 45 min, 10 µM eserine failed to re-

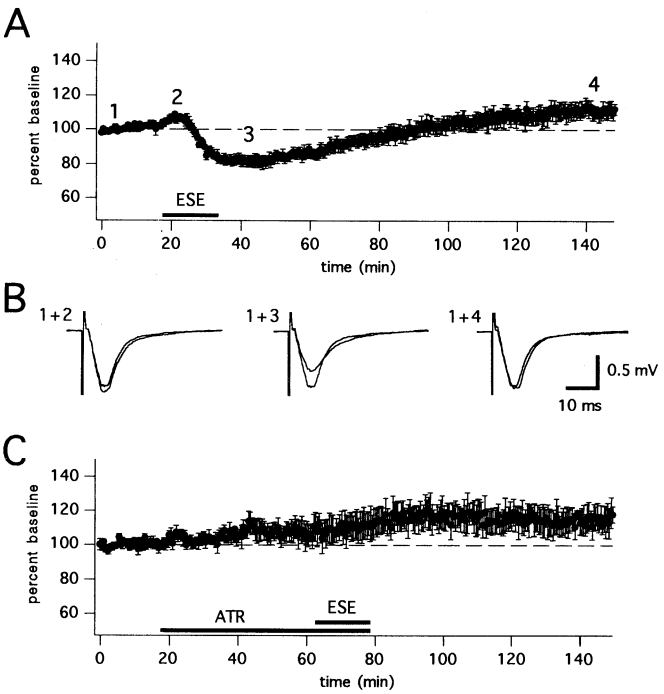
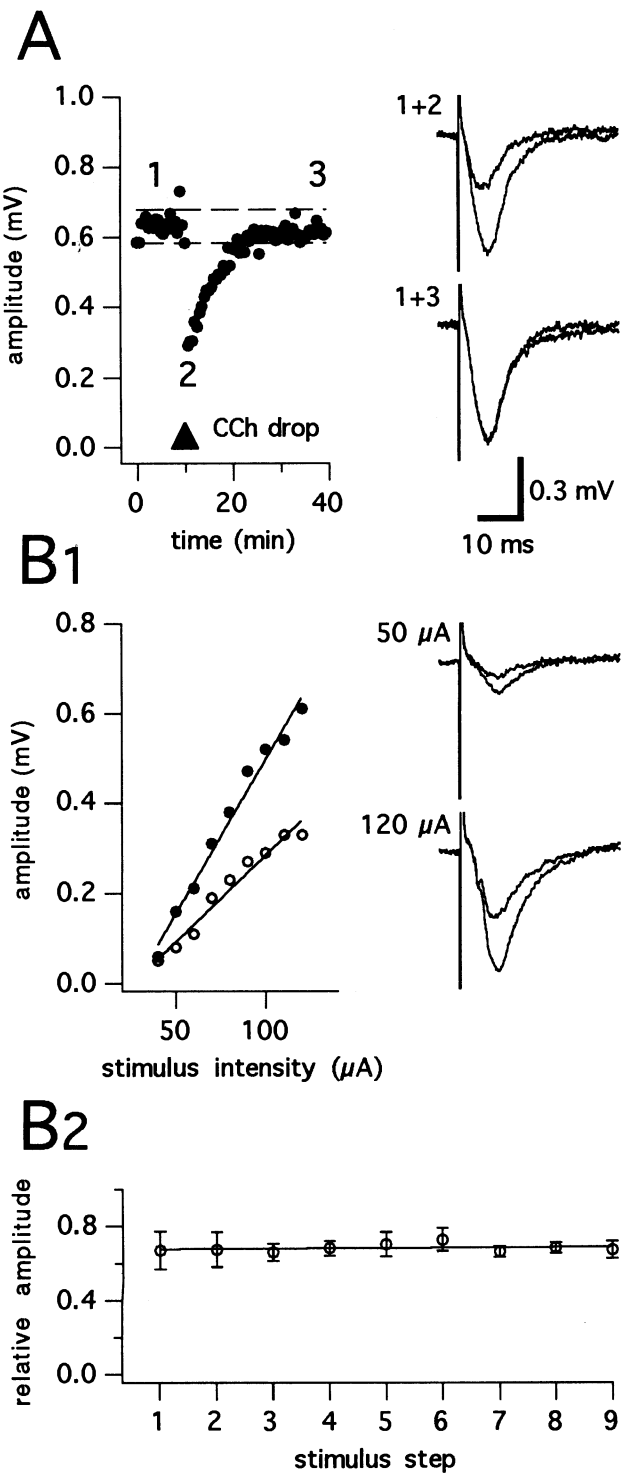


Fig. 1. Acetylcholinesterase inhibition results in a depression of responses evoked in horizontal pathways. A, timecourse of the effects of bath application of 10 µM eserine on field potentials. Shown are means ±SEM (n=6), bar denotes time of eserine (ESE) bath-application. B, superpositions of examples of responses (10 trial averages) from a representative experiment, recorded at times indicated in A. C, plot of the response amplitude (means ± SEM, n=4), recorded during 10 µM atropine (ATR) and subsequent 10 µM eserine (ESE) application (bars).

duce field response (n=4). However, the application of both atropine and eserine induced an increase in response amplitude (on average 115% of control, n=4), exceeding the duration of drug application by at least 70 min (see Fig. 1B).



Application of the exogenous acetylcholine agonist carbachol (CCh) reversibly depressed layer II/III horizontal field responses. Figure 2A shows an example of the effect of CCh (1 mM) which was drop-applied close to the recording site and induced about 50% reduction of the response. Due to a high concentration of the drug, the magnitude of this effect is likely to represent the maximum possible reduction. Bath application of CCh resulted in a dose-dependent decrease of field response. The application of 0.5 μM CCh reduced field potentials to 95±2% of control (n=8) whereas 5 μM CCh reduced responses to 70±1% of control (n=16). Figure 2B1 shows an example of a particularly prominent effect of 5 μM CCh on responses evoked by a series of increasing stimulus intensities. This figure demonstrates that the relative reduction of response by CCh was independent of stimulus intensity, indicating that the transmission in all classes of activated fibers was affected by CCh to similar extent. This finding was repeated in 5 identical experiments (see Fig. 2B2). In these experiments, mean response during 5 μM CCh application, measured as a ratio of slopes of linear fits to data obtained in CCh and control (see Fig. 2B1), was reduced to 68±4% of control (n=5). The application of 100 μM CCh did

Fig. 2. Carbachol (CCh) -induced depression of field potentials in horizontal pathways. A, left panel - timecourse of the effect of a drop of CCh (1 mM) applied from a pipette by touching the slice surface within 100 μm from the recording microelectrode at a time indicated by the triangle. Broken lines indicate ±2 SD over baseline. Superpositions of responses (3 trial averages) recorded at times indicated at the plot are shown to the right. B, effect of CCh is not dependent on stimulus intensity. B1, example of the effect of bath-applied CCh (5 μM) on responses evoked by stimuli of different intensities. Left panel: filled and open symbols indicate amplidude of responses recorded in control solution and in CCh, respectively. Continuous lines represent a linear fit to the data. To the right - superpositions of responses evoked during control period and after CCh application at low (50 μA) and high (120 μA) stimulus intensities (4 trial averages). Data from the same experiment. B2, mean (±SEM) amplitude of responses recorded in the presence of CCh plotted as a fraction of control response amplitude (n=5). Stimulus intensity was varied between just suprathreshold to submaximal in nine equal steps (see an example in B1). Continuous line represents a linear fit to the data and its slope is not significantly different from zero.

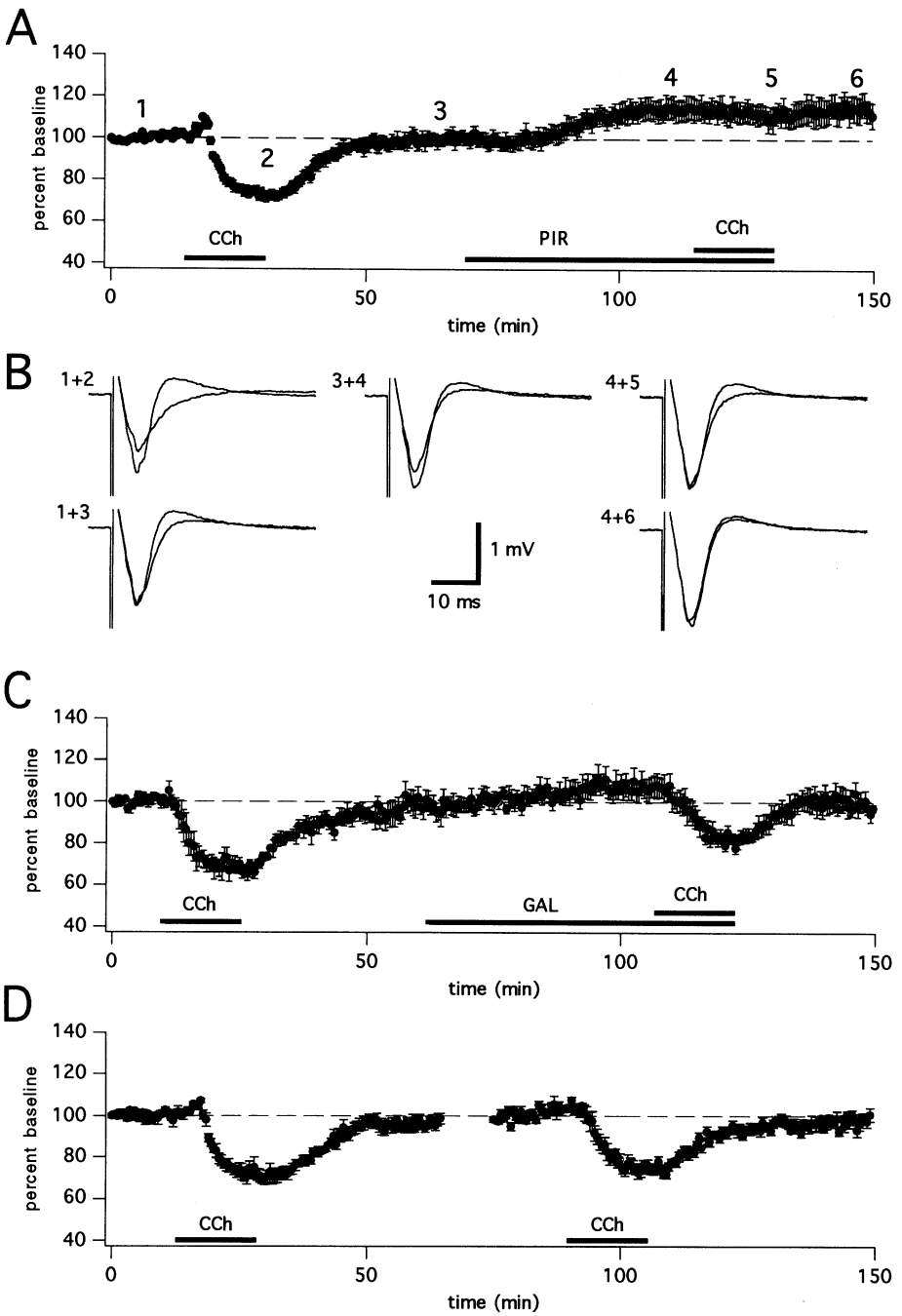


Fig. 3. Carbachol-induced depression of horizontal responses is blocked by pirenzepine but not by gallamine. A, carbachol (CCh, 5 μM) was applied for 15 min and then washed out. Next, pirenzepine (PIR, 3 μM) was bath-applied and after stabilization of responses the application of 5 μM CCh was repeated. In the presence of pirenzepine no CCh - induced depression was observed (compare B: 1+2 vs. 4+5). Note the response increase due to pirenzepine (see B: 3+4). Bars denote times of drug applications. Shown are means ±SEM (n=7). B, superpositions of examples of field potentials from a representative experiment recorded at times indicated by numbers in A (averages of 10 sweeps). C, carbachol (CCh, 5 μM) was applied first, and after washout and preincubation in gallamine (GAL, 10 μM) the application of CCh was repeated. Note the reduction of response by CCh in the presence of gallamine (n=5). D, repeated application of CCh induced similar effects on field response amplitude (n=4). The discontinuity in data plot is due to the adjustment of data recorded just before the second CCh application to 100% in order to facilitate comparison.

not induce much larger reduction (to: 57 - 60% of baseline, n=2). In contrast to the effects of low concentration of the drug which were readily reversible (see Fig. 3), the responses recorded in 100 μM CCh did not return to control levels after 1-2 h of washout.

In order to characterize the type of receptor mediating the reduction of field responses, the application of CCh was attempted in the presence of pirenzepine, an M1 muscarinic receptor antagonist or gallamine, an M2 receptor antagonist. In this set of experiments, control application of 5 μM CCh for 15 min was followed by washout lasting 40 min, then the antagonists were added to the incubation solution for 45 min and CCh application was repeated. As shown in Fig. 3A, 3 μM pirenzepine completely blocked the effect of CCh (n=7). On the

contrary, in the presence of 10 μM gallamine the effects of 5 μM CCh resembled control applications (n=5, see Fig. 3C). However, CCh-induced reduction was smaller in the presence of gallamine (23±2% reduction, see Fig. 3C) than during control application in same slices (33±3% reduction) and the difference was statistically significant (P<0.05, Wilcoxon signed rank test). Separate control experiments (see Fig. 3D) proved that repeated CCh application resulted in a similar degree of response reduction by the first and the second drug delivery (29±3% vs. 25±2%, n=4). After addition of pirenzepine to the incubation medium a 15% response increase was observed (see Fig. 3A, B: 3+4). This effect was not observed during perfusion with gallamine (see Fig. 3C).

In order to determine the site of action of CCh, its effect on responses evoked by paired-pulses has been tested (Hasselmo and Bower 1992, Auerbach and Segal 1996). Paired-pulse stimulation (40 ms interval) of motor cortex horizontal connections resulted in a facilitation of the response to the second pulse. Figure 4 shows that in the presence of 5 μM carbachol, despite of an overall reduction of responses, the ratio of amplitudes of the second to the first response (termed facilitation ratio) was increased over a broad range of stimulus intensities.

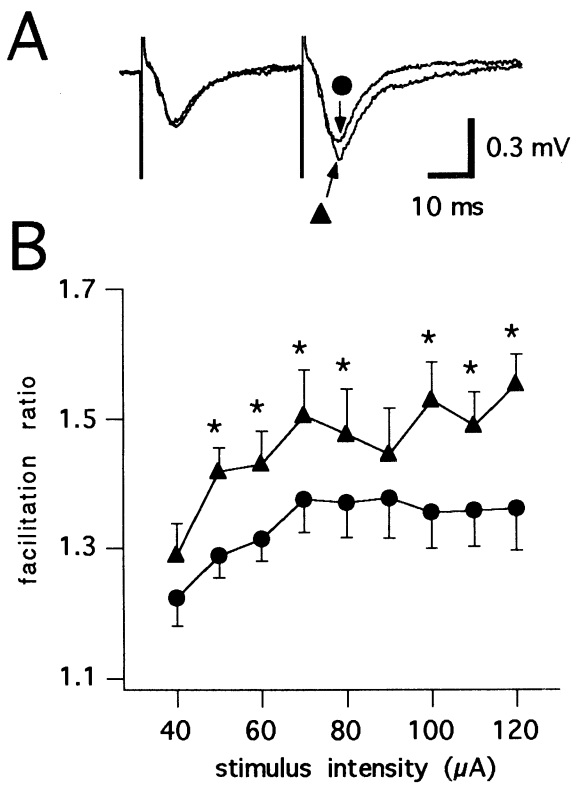


Fig. 4. Increase of paired-pulse facilitation in 5 μM carbachol. A, examples of paired-pulse-evoked field responses obtained during control (circle) and in the presence of CCh (triangle), in which amplitudes of the responses to the first pulse matched approximately each other (averages of 4 trials). B, increase of paired-pulse facilitation over a range of stimulus intensities. Filled circles and triangles denote data obtained during control and in the presence of 5 μM CCh, respectively (means ±SEM, n=11). Asterisks mark statistically significant differences (P<0.05, two-tailed t-test).

DISCUSSION

The results obtained in the course of this study indicate that endogenous and exogenous cholinergic agonists may transiently depress excitatory synaptic transmission within layer II/III horizontal connections of rat motor cortex in the *in vitro* slice preparations. The action of endogenous acetylcholine on neuronal membrane properties has previously been observed in slices isolated from auditory (Metherate et al. 1992, Cox et al. 1994) and somatosensory cortex (Benardo 1993). The influence of muscarinic cholinergic activation on neocortical synaptic transmission, where investigated, has been shown to result in a reduction of the synaptic responses. It has recently been reported that eserine application depressed glutamatergic and GABA-ergic synaptic transmission in vertical (layer VI-layer III) connections in the auditory cortex and this effect depended on muscarinic receptors (Metherate and Ashe 1995). Exogenous cholinergic agonists depress excitatory postsynaptic potentials evoked by local intracortical stimulation in cingulate (McCormick and Prince 1986) and prelimbic cortical neurons (Vidal and Changeux 1993), and those evoked by white matter stimulation in visual cortical neurons (Murakoshi 1994). Similar effects were observed in synaptic connections formed by intrinsic fibers within piriform cortex (Hasselmo and Bower 1992) and in the hippocampus (Yamamoto and Kawai 1967, Williams

and Johnston 1988, Sheridan and Sutor 1990). Thus, present data are consistent with results obtained from other cortical areas and, together with an earlier communication (Hess 1991), indicate that muscarinic modulation of synaptic transmission in horizontal and vertical connections within rat motor cortex resembles the effects observed in other cortical synaptic systems. One notable difference is that, in contrast to the hippocampal CA1 area (Auerbach and Segal 1994, 1996), no long-lasting increase of responses evoked in horizontal pathways was induced by low (0.5 μM) concentration of carbachol. This effect might reflect a generally lower probability of evoking long-term increases of synaptic efficacy in the motor cortex than in the hippocampus (Hess and Donoghue 1994). It has been reported that incubation of hippocampal slices in high (50-200 μM) concentration of carbachol induced oscillatory activity in the theta range (Konopacki et al. 1987). Such an effect was not observed in motor cortical slices.

The reduction of field potentials by CCh in the present study was blocked completely by a low dose (3 μM) of the M1 receptor antagonist, pirenzepine, which is suggestive of the involvement of this type of muscarinic receptor. This conclusion is further strenghtened by the observation that gallamine, the antagonist of M2 receptors, applied at a higher concentration (10 μM) only slightly influenced the occurrence of CCh-induced reductions of field potentials. These results are consistent with data obtained from piriform cortex (Hasselmo and Bower 1992) and the hippocampal cortex (Sheridan and Sutor 1990). However, in a recent study, the involvement of M3 receptors in CCh-induced depression has been reported in the hippocampus (Auerbach and Segal 1996). Based on the observation that the depressive effect of CCh on responses was more sensitive to unspecific muscarinic antagonists, such as atropine and scopolamine, than to pirenzepine, it has been suggested that M2 receptors mediated the reduction of postsynaptic potentials in the prelimbic cortex (Vidal and Changeux 1993). These discrepancies might be explained by the fact that a different cortical region and synaptic system was investigated in the present study. In the visual and prefrontal cortices, m2 muscarinic receptor proteins (presumably corresponding to pharmacologically defined M2 receptors, see Brann et al. 1993) have been found in presynaptic terminals using specific antibodies while m1 and m2 receptors have been located on postsynaptic membranes (Mrzljak et al. 1993).

An increase of the response amplitude by about 15% was observed during incubation in the presence of pirenzepine. A slight increase was observed after atropine application as well. These effects may suggest that synaptic transmission in normal incubation conditions remains under the influence of continuous activation of M1 receptors, resulting in a slight suppresion of evoked responses. Similar observations have also been made in preparations of auditory cortex (Metherate and Ashe 1995).

Vidal and Changeux (1993) observed an increase in the amplitude of postsynaptic potentials by 23% after activation of nicotinic receptors in 14% of investigated cells. While in the present study the main effect of the reduced acetylcholine breakdown by means of eserine application was the reduction in response amplitude, the application of eserine after preincubation in atropine and the washout of eserine resulted in a small increase of responses by 11-14% over baseline. One interpretation of this result is that acetylcholine acting on nicotinic receptors might enhance synaptic transmission in a subpopulation of synapses comprising a small fraction of all activated fibers. Intracellular recording combined with low intensity stimulation will be required to test whether specific classes of synapses are modulated by nicotinic receptors in rat motor cortex.

While in the present study the influence of CCh on the evoked responses was found to be independent of stimulus intensity, Metherate and Ashe (1995) observed a different degree of depression by CCh of a glutamatergic component of field potentials evoked by weak and strong stimuli in the vertical (intracolumnar) connections of auditory cortex. This apparent discrepancy may be explained

by the fact that the peak and the late phase of the field potentials recorded in that study were strongly influenced by the GABA-ergic component. A high level of activity within a cortical column engages inhibitory synaptic transmission to a much higher degree than weak activation, the effect reflected in the occurrence of a positively-directed wave after initial negativity at high stimulus intensities (Metherate and Ashe 1995). It seems likely that the simultaneously occurring, CCh-induced reduction of the inhibitory component of the field potential obscured the real, larger effect of CCh application on the excitatory component of the field. On the contrary, the activation of horizontal pathways within motor cortex usually results in a generation of a monophasic, negative-going waveforms if the distance between stimulating and recording microelectrodes is longer than 0.5 mm, indicative of a relatively smaller contribution of inhibitory transmission to the recorded potentals (see also Hess et al. 1996). It is likely that in these conditions the peak of the field potential is not significantly contaminated by inhibitory components. For this reason, in horizontal pathways, the extent of the influence of CCh on the field potential is not dependent on its amplitude.

Paired-pulse facilitation, a form of short-term (less than 1 s) synaptic plasticity is generally thought to represent a presynaptic phenomenon (Zucker 1989). Calcium imaging studies have demonstrated that in the hippocampal cortex, the facilitation of the response to the second pulse of a pair is due to a transient increase in the residual Ca^{2+} level in presynaptic terminals (Hess and Kuhnt 1992, Wu and Saggau 1994). It is widely assumed that experimental manipulations affecting paired-pulse facilitation are indicative of a presynaptic location of underlying mechanism. A postsynaptic mechanism would influence both the first and the second response equally (Harris and Cotman 1985). This approach has been used in studies on long-term potentiation (e.g. Schulz et al. 1994, Wu and Saggau 1994) and on the action of cholinergic agonists (Hasselmo and Bower 1992, Auerbach and Segal 1996). In agreement with the latter studies, we found that despite an overall decrease of responses, CCh application produced a small but consistent relative response increase to the second pulse of a pair. The observed type of the change in shape of field responses may suggest that, alternatively, a larger reduction of inhibitory than excitatory transmission in the presence of CCh (Sugita et al. 1991, Metherate and Ashe 1995) could contribute to the relative increase of the response to the second pulse of a pair. However, this interpretation is not supported by the fact that the facilitation ratio increased to the similar extent over a wide range of stimulus intensities. As discussed above, the contribution of synaptic inhibition to field potentials evoked in horizontal pathways is small, especially at low stimulus intensities. If inhibition played a significant role in the observed effects, a difference would be expected, depending on the stimulus strength. Therefore, it is more likely that the receptors mediating the depressive effect of CCh on excitatory synaptic transmission are located presynaptically. Similar conclusions have been reached in studies concerning other neocortical areas (Vidal and Changeux 1993, Murakoshi 1994), piriform cortex (Hasselmo and Bower 1992), hippocampal CA1 area (Sheridan and Sutor 1990) and such structures like lateral amygdala, nucleus accumbens and striatum (Sugita et al. 1991). The existence of m2 muscarinic receptor proteins has been documented in excitatory presynaptic terminals in primate neocortex using specific antibodies (Mrzljak et al. 1993).

The results of this study indicate that acetylcholine may modulate synaptic transmission in horizontal connections through muscarinic receptors of the M1 subtype, most likely located presynaptically on glutamatergic terminals in a similar way to that observed across various brain areas. Thus, data suggest that this effect represents a general neuromodulatory mechanism of glutamatergic transmission. The question remains, whether the effect of bath-applied carbachol has a physiological relevance. However, the influence of endogenous acetylcholine on normal excitatory and inhibitory synaptic transmission is likely because of the widespread diffuse cholinergic innervation of

neocortex arising from basal forebrain (Lysakowski et al. 1989). It has been observed that apart from typical synaptic contacts (e.g. Beaulieu and Somogyi 1991) cholinergic fibers also form nonsynaptic appositions with neurons (Mrzljak et al. 1995). These structures have frequently been found in close proximity to asymmetric, presumably excitatory glutamatergic synapses. Changes in the level of acetycholine release have been observed, depending on the behavioral state of the animals (Jimenez-Capdeville and Dykes 1996). Therefore, it is conceivable that physiological release of acetylcholine might tune excitatory synaptic transmission within various neocortical areas and modulate the potential for synaptic plasticity.

ACKNOWLEDGEMENTS

The comments of Dr. J.P. Donoghue on earlier version of this manuscript are greatly appreciated. This research was supported by the State Committee for Scientific Research (KBN) grant 6P04C 053 08, and Howard Hughes Medical Institute grant 75195-543-101. Pilot experiments were supported in part by NIH grant NS-22517.

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Received 1 June 1996, accepted 4 October 1996