Muscarinic Receptor Activation Induces Depolarizing Plateau Potentials in Bursting Neurons of the Rat Subiculum

HIROTO KAWASAKI,1 CARMELA PALMIERI,1,2 AND MASSIMO AVOLI1
1Montreal Neurological Institute and Departments of Neurology and Neurosurgery and of Physiology, McGill University, Montreal, Quebec H3A 2B4 Canada; and 2Centro per l’Epilessia, Ospedale San Paolo, Università degli Studi di Milano, 20142 Milan, Italy

Muscarinic receptor activation induces depolarizing plateau potentials in bursting neurons of the rat subiculum. J. Neurophysiol. 82: 2590–2601, 1999. Acetylcholine functions as a neuromodulator in the mammalian brain by binding to specific receptors and thus bringing about profound changes in neuronal excitability. Activation of muscarinic receptors often results in an increased excitability of cortical cells. It is, however, unknown whether such an action is present in the subiculum, a limbic structure that may be involved in cognitive processes as well as in seizure propagation. Most rat subicular neurons are endowed of intrinsic membrane properties that make them fire action potential bursts. Using intracellular recordings from these bursting cells in a slice preparation, we report here that application of the cholinergic agonist carbachol (CCh, 30–100 μM) to medium containing ionotropic excitatory amino acid receptor antagonists reduces burst-afterhyperpolarizations (burst-AHPs) and discloses depolarizing plateau potentials that outlast the triggering current pulses by 140–2,800 ms. These plateau potentials appear with CCh concentrations >50 μM and are dependent on the resting membrane potential and on the intensity/duration of the triggering pulse; are recorded during application of tetrodotoxin (1 μM, n = 5 neurons); but are markedly reduced by replacing 82% of extracellular Na+ with equimolar choline (n = 6). Plateau potentials also are abolished by Cd2+ (2 mM; n = 5) or Ca2+ (1 mM; n = 2) application and by recording with electrodes containing the Ca2+-chelator bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (0.2 M; n = 6). CCh-induced burst-AHP reduction and plateau potentials are reversed by the muscarinic antagonist atropine (0.5 μM, n = 7). In conclusion, our findings demonstrate a powerful muscarinic modulation of the intrinsic excitability of subicular bursting cells that is predominated by the appearance of plateau potentials. These changes in excitability may contribute to physiological processes such as learning or memory and play a role in the generation of epileptiform depolarizations. We propose that, as in other limbic structures, muscarinic plateau potentials in the subiculum are mainly due to a Ca2+-dependent nonselective cationic conductance.

INTRODUCTION

The cholinergic system plays a crucial role in modulating cortical (and in particular hippocampal) functions including processes such as learning and memory (Ashe and Weinberger 1991; Dunnett and Fibiger 1993; Huerta and Lisman 1993; Shen et al. 1994; Winkler et al. 1995). Cholinergic actions may be also involved in the physiopathogenesis of epileptic discharges as suggested by the ability of some cholinergic agents to induce limbic seizures and histopathological changes resembling those seen in patients with temporal lobe epilepsy (Dickson and Alonso 1997; Liu et al. 1994; Nagao et al. 1996; Turski et al. 1989).

Cholinergic stimulation of cortical neurons, including those located within the hippocampal formation, results in excitatory effects that are mediated mainly through the activation of muscarinic receptors (see for review, Krnjević 1993; McCormick 1993). Cholinergic innervation is present in the subiculum, which is a major synaptic relay station between the hippocampus proper and several limbic structures that are involved in cognitive processes (Amaral and Witter 1989; Lopes da Silva 1990). For instance, subicular cells recorded from freely moving animals generate “location specific” firing patterns; this indicates a possible contribution of this region to spatial learning (Barnes et al. 1990; Sharp and Green 1994). Subicular neurons may be also involved in the spread of seizure activity within the limbic system (Lothman et al. 1991).

To date little is known about the effects of cholinergic agents in the subiculum. Hence in this study, we characterized the changes induced by the nonhydrolysable cholinergic agonist carbachol (CCh) on the intrinsic excitability of bursting neurons of the rat subiculum. Here we report that CCh induces powerful excitatory effects that are mediated through the stimulation of muscarinic receptors and include the well-known decrease of K+ conductances underlying afterhyperpolarizations (AHPs) and the appearance of depolarizing plateau potentials that are contributed by a Ca2+-dependent nonselective cationic conductance. A preliminary account of these results has appeared (Kawasaki and Avoli 1996).

METHODS

Brain slices were obtained from male Sprague-Dawley rats (200–300 g), following standard procedures (Nagao et al. 1996). In brief, rats were decapitated under halothane anesthesia. Their brain was removed quickly from the skull and immersed in cold (1–3°C) artificial cerebrospinal fluid (ACSF). Horizontal slices (450–500 μm thick) containing the whole subicular complex were cut with a vibroslice apparatus and were transferred to a tissue chamber where they lay between oxygenated ACSF and humidified gas (95%O2,5%CO2) at 32–33°C. Slices were perfused continuously with oxygenated ACSF composed of (in mM) 124 NaCl, 2 KCl, 1.25 KH2PO4, 2 MgSO4, 2 CaCl2, 26 NaHCO3, and 10 glucose (pH 7.4). In all experiments, the ACSF contained the ionotropic excitatory amino acid receptor antagonists 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX, 10 μM) and 3-((±)-2-carboxytopiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 20 μM) to abolish ionotropic excitatory transmission.

Depending on the experimental procedure, the following com-
pounds also were added to the ACSF: (+)-alpha-methyl-4-carboxyphenylglycine (MCPG, 1 mM), 4-aminoypyridine (4AP, 5 mM), atropine (0.5 μM), bicuculline methiodide (BMI, 10 μM), CCh (30–100 μM), P3-amino-propyl, P-diethoxymethylphosphonic acid (CGP-35348,1 mM), tetraethylammonium (TEA, 10 mM), tetrodotoxin (TTX, 0.5 μM), and N-tubocurarine (N-TC, 1 μM). In some experiments, NaCl in the ACSF was replaced with equimolar choline. When the Ca2+ channel blockers Cd2+ (2 mM) or Cd2+ (1 mM) were used, KH2PO4 was omitted, MgSO4 was replaced with MgCl2, and KCl was increased from 2 to 3.25 mM. Chemicals were acquired from Tocris Cookson or Sigma, except CGP-35348 which was a kind gift of Novartis.

Intracellular recordings were made with sharp-electrodes pulled from capillary tubes and back filled with one of the following solutions: 3 or 4 M K-acetate (resistance = 80–120 MΩ); 3 M K-acetate + 0.2 M of the Ca2+ chelator bis(2-aminophenoxo)ethane-N,N,N′,N′-tetraacetic acid (BAPTA; resistance = 100–150 MΩ); or KCl (resistance = 60–100 MΩ). Signals were fed to a high-impedance amplifier (Axoclamp 2) with an internal bridge circuit for passing intracellular current. The bridge was monitored carefully throughout the experiments and adjusted as required. Whenever necessary the resting membrane potential (RMP) was maintained at the same value as in control by injecting steady intracellular current. Signals were displayed on an oscilloscope and on a Gould chart recorder, and were acquired by means of a computerized system (DigitData 1200, pClamp 6, Axon Instruments). A bipolar stainless steel electrode was used at times to deliver extracellular stimuli (0.5–1.8 nA; 90 μs) to establish whether excitatory synaptic transmission was effectively abolished by CNQX and CPP.

Electrophysiological parameters were measured as follows: RMP after withdrawal from the cells; input resistance (Ri) from the maximum voltage changes attained during hyperpolarizing current pulses (duration = 100–200 ms, intensity less than ~0.5 nA); and action potential amplitude from the baseline. All quantitative results are expressed as means ± SD, and n indicates the number of neurons analyzed under each experimental procedure. The results were compared by using the paired or unpaired Student’s tests and were considered significantly different if P < 0.05.

RESULTS

Characteristics of the subicular bursting neurons in control medium

The data obtained in this study are based on 103 subicular neurons that were identified as bursting-firing cells according to the criteria reported in previous studies (Mason 1993; Mattia et al. 1993, 1997a,b; Stuart and Wong 1993; Taube 1993) (see also following text). The membrane properties of a sample of these neurons recorded with K-acetate-filled microelectrodes in control ACSF containing CNQX and CPP were ~65.1 ± 7.0 mV (n = 36) for the RMP, 42.4 ± 9.9 MΩ for the Ri (n = 34), and 73.4 ± 3.2 mV for the action potential amplitude (n = 32). Several types of membrane rectification, including a Cs+-sensitive inward rectification in the hyperpolarizing direction (cf. Mattia et al. 1997a), were observed during injection of intracellular current pulses (Fig. 1, controls).

Subicular cells responded to pulses of depolarizing current (10–120 ms, <0.7 nA) injected at RMP with a burst of three to five action potentials that was terminated by a burst-AHP with duration = 115.3 ± 31.3 ms (n = 13) and peak amplitude = 7.5 ± 3.1 mV (n = 13) (arrows in Fig. 1, A and B, control). Single action potentials could occur on termination of the burst-AHP (Fig. 1B, control). We have reported that this bursting mode of firing is contributed mainly by a voltage-gated, Na+ conductance, whereas the burst-AHPs are reduced by Ca2+-channel blockers (Mattia et al. 1993, 1997a,b).

Effects induced by carbachol on the bursting response

Addition of CCh (30–100 μM) to control ACSF caused a 5.4 ± 3.3 mV (n = 34) steady membrane depolarization that was at times associated with spontaneous action potential discharge (data not illustrated). These effects could be accompanied by an augmentation of the membrane input resistance. Such an increase also was appreciated when the membrane potential was brought back to control values, and in these cases amounted to 17.0 ± 11.5% (n = 14; Fig. 1A).

In the presence of CCh, subicular neurons continued to generate action potential bursts in response to intracellular injection of depolarizing current pulses. These bursts occurred at the depolarized RMP induced by CCh (not illustrated) as well as when the RMP was made similar to that recorded under control conditions (Fig. 1, CCh). However, CCh decreased and eventually abolished the burst-AHP. Such an effect was paralleled by prolongation of the depolarizing envelope underlying the burst and the appearance of tonic action potential firing that ‘replaced’ the burst AHP (Fig. 1, CCh). These action potentials lacked the fast AHP seen under control conditions (arrow–head in Fig. 1B, control), and had duration significantly greater than what observed under control conditions (half-width = 3.7 ± 1.2 ms vs. 2.1 ± 0.4 mV, n = 11; Fig. 1, B and C), although such an increase could be due to the depolarized membrane level from which tonic firing action potentials arose in the presence of CCh. As illustrated in Fig. 1C (top), CCh did not cause any measurable change in the shape or duration of the initial action potential burst.

The action potential discharge induced by an intracellular depolarizing pulse in the presence of CCh, was followed by a slow depolarization (duration = 40–90 ms) that outlasted the termination of the pulse (Fig. 1, A and B, double arrow–heads in CCh samples) and eventually could lead to the appearance of a depolarizing plateau potential (see following text). CCh did not cause any measurable change in the Cs+-sensitive inward rectification in the hyperpolarizing direction but caused a significant decrease of the slow rebound depolarizations that followed the hyperpolarizing current pulses under control conditions (Fig. 1A, asterisk in control).

Action potential firing induced by intracellular depolarizing pulses during CCh application was abolished by TTX (n = 4), along with the slow, postpulse depolarization. As shown in Fig. 1A (CCh + TTX), pulses of large depolarizing current injected during application of CCh + TTX failed to elicit slow regenerative events as reported in hippocampal neurons during blockade of voltage-gated Na+ channels where they presumably reflect the activation of a high-threshold Ca2+-conductance (Wong and Prince 1978). The effects induced by CCh could be reversed on wash with control ACSF (Fig. 1B, wash).

CCh-induced plateau potentials

CCh concentrations >50 μM also caused the appearance of plateau potentials that outlasted the termination of the intracellular depolarizing pulse by 160–2,800 ms (maximal duration = 940 ± 314 ms, n = 19) and attained peak amplitudes of 8.1–34 mV (20.6 ± 10.2 mV, n = 17). Sustained, tonic firing
of action potentials at 15–50 Hz and/or action potential bursts, occurred during these plateau potentials (Figs. 2–4) that were terminated by a slow repolarization often resulting in an AHP with duration = 80–530 ms (* in Figs. 2C and 4B). The plateau potential magnitude (which includes duration, amplitude, and ability to trigger action potential discharge) was a direct function of the duration and/or intensity of the triggering intracellular depolarizing pulse (Fig. 2). However, the duration of the plateau potentials often became shorter with high-intensity pulses (Fig. 2B). Plateau potentials recorded in any given neuron in response to current pulses of fixed intensity and duration had relatively stereotyped characteristics with stimuli delivered at intervals >60 s (Fig. 3A, 120-s interval). By contrast, intracellular pulses repeated at shorter intervals made the plateau potential decrease in duration and/or fail to occur (Fig. 3A, 60- and 10-s intervals). These “refractory” features are quantified for one bursting neuron in the plots of Fig. 3B.

The plateau potential duration and amplitude (as well as the occurrence of action potential firing) were voltage dependent. Small changes in membrane potential (i.e., ±2 mV from an RMP = −68 mV) increased or decreased the magnitude of the plateau potentials induced by depolarizing current pulses of fixed intensity and duration (10 ms, +0.5 nA, stimulation interval = 120 s), even though this procedure did not modify the initial action potential burst (Fig. 4A). In addition, injection of a brief (duration = 10–20 ms) hyperpolarizing pulse after the initial depolarizing command made the plateau potentials decrease in duration and eventually disappear (Fig. 4B). Shaping a hyperpolarizing pulse by changing its termination modalities also could abort the plateau potential and give origin to recurrent bursts (Fig. 4C).
Pharmacological features of the changes in excitability induced by carbachol

The effects induced by CCh were not influenced by the nicotinic receptor antagonist d-TC (1 μM, n = 3) but were abolished by the muscarinic receptor antagonist atropine (0.5–1 μM; n = 7). As illustrated in Fig. 5A, addition of atropine to ACSF containing CCh made the burst-AHP reappear, whereas the input membrane resistance returned to values that were similar to those seen under control conditions. Moreover, atropine abolished in all experiments (n = 7) the CCh-induced plateau potentials (Fig. 5B).

The ability of intracellular depolarizing pulses to induce depolarizing plateau potentials during application of CNQX and CPP indicates that muscarinic plateau potentials are not contributed by the activation of ionotropic excitatory amino acid receptors. We also ruled out the unlike participation of inhibitory conductances to the generation of the plateau potentials by using BMI (10 μM) and CGP-35348 (1 mM). Concomitant application of these GABA<sub>A</sub> and GABA<sub>B</sub> receptor antagonists did not modify the ability of four subicular cells to generate plateau potentials (data not illustrated, but see Fig. 6A). Finally, we analyzed the possible role played by metabotropic glutamate receptors by using MCPG (1 mM; n = 4). As illustrated in Fig. 5C, the CCh-induced plateau potentials were not influenced by this pharmacological procedure.

Ionic mechanisms underlying the carbachol-induced plateau potentials

CCh-induced depolarizing plateau potentials also were studied with KCl-filled electrodes, a recording procedure that increases [Cl<sup>−</sup>], and thus shifts the reversal potential of Cl<sup>−</sup>-mediated conductances overtime. All subicular neurons recorded with KCl-filled electrodes (n = 6) generated spontaneous, “asynchronous” depolarizing potentials with amplitude = 8–15 mV and interval of occurrence ranging between 80 and 250 ms (Fig. 6A, *). Intracellular depolarizing current pulses evoked in these neurons plateau potentials with characteristics (i.e., duration and associated action potential firing as well as presence of postplateau potential AHP) that were superimposable to those observed in a larger neuron sample.
with K-acetate-filled recording electrodes. Moreover, the duration and/or amplitude of the plateau potentials studied with KCl-filled electrodes did not change over time (≤85 min, Fig. 6A). In two experiments application of BMI abolished the spontaneous, asynchronous depolarizations, thus indicating that they represented GABAA-receptor-mediated postsynaptic potentials. This effect was not accompanied by any change in the amplitude or duration of the depolarizing plateau potentials. Hence, these findings indicate a negligible contribution of Cl\(^{-}\)-mediated conductances to the generation of depolarizing plateau potentials recorded in the presence of CCh.

It has been shown by Colino and Halliwell (1993) that CCh potentiates a Cs\(^{+}\)-sensitive, hyperpolarization-activated cationic conductance (so-called \(I_Q\) or \(I_h\)) in CA1 pyramidal cells. This current contributes to the hyperpolarizing sag recorded in subicular bursting cells under control conditions (Mattia et al. 1997a). Hence we investigated the effects induced by extracellular application of Cs\(^{+}\) (2 mM \(n = 6\)) on the plateau potentials generated by subicular bursting cells during muscarinic receptor activation. As shown in Fig. 6Ba, Cs\(^{+}\) abolished the time-dependent inward rectification in the hyperpolarizing direction, increased the membrane input resistance, and further decreased the slow, rebound depolarizations that follow the termination of hyperpolarizing current pulses (Fig. 6Ba, arrow). These effects were not accompanied by any change in the ability of subicular bursting cells to generate plateau potentials or their magnitude (Fig. 6Bb). In two of six neurons Cs\(^{+}\) decreased the amplitude of the postdepolarizing plateau potential AHP (Fig. 6B, arrows).

**Ca\(^{2+}\) dependence of plateau potentials**

Next, we investigated the Ca\(^{2+}\) dependence of the CCh-induced depolarizing plateau potentials. In a first series of experiments, we applied the inorganic Ca\(^{2+}\) channel blockers Co\(^{2+}\) (\(n = 5\)) or Cd\(^{2+}\) (\(n = 2\)) during perfusion with CCh-containing ACSF. Depolarizing plateau potentials disappeared in all experiments following blockade of Ca\(^{2+}\) channel activity while action potential bursts continued to be elicited by brief current pulses (Fig. 7A). Action potential bursts recorded in the presence of either Co\(^{2+}\) or Cd\(^{2+}\) were followed by a depolarizing afterpotential with duration \(= 30 – 60\) ms (Fig. 7A, ↓). Moreover, intracellular pulses that were unable to trigger action potential bursts during CCh application did so after addition of either Co\(^{2+}\) or Cd\(^{2+}\) (Fig. 7A, 0.3-nA samples).

In a second series of experiments, we used electrodes filled with K-acetate and the Ca\(^{2+}\) chelator BAPTA to record the activity generated by 6 bursting cells in the presence of CCh. Shortly after the impalement, at a time when the neuron RMP had stabilized, all neurons recorded with BAPTA electrodes generated CCh-induced plateau potentials (duration \(= 940 ±\)
threshold $\text{Ca}^{2+}$ reflect the inability of subicular bursting cells to produce high-$\text{Ca}^{2+}$ spikes in the absence of functional voltage-gated $\text{Na}^{+}$ channels (Mattia et al. 1997a). Indeed, high-threshold $\text{Ca}^{2+}$ spikes can be recorded in subicular bursting neurons when TTX is applied concomitantly with the $\text{K}^{+}$ channel blocker TEA (Mattia et al. 1997a,b). Hence, we analyzed the involvement of $\text{Na}^{+}$-mediated electrogenesis in the occurrence of CCh-induced plateau potentials by pretreating subicular neurons with extracellular application of TEA (10 mM).

As illustrated in Fig. 8A (inset), TEA modified the action potential burst recorded in control ACSF into a complex intracellular potential with a predominant slow event which represented a presumptive $\text{Ca}^{2+}$ spike (↓ in Fig. 8A, inset). The depolarizing pulse-triggered responses recorded during TEA application from 10 neurons never outlasted the termination of the depolarizing command by $>50$ ms and were terminated by an AHP (postspike AHP) with duration = $107 \pm 38$ ms and amplitude = $9.4 \pm 3.2$ mV (* in Fig. 8, inset). Similar data also were obtained in three neurons during concomitant application of TEA (10 mM) and 4AP (5 mM). Application of CCh to these neurons (12/12) blocked the depolarizing plateau potentials. These events had maximal duration = $722 \pm 135$ ms ($n = 10$) and were associated with both fast and slow regenerative events (Fig. 8, A and B, TEA + CCh). Further application of TTX to ACSF containing TEA and CCh abolished in 5/5 neurons the fast events but did not interfere with the ability of these cells to generate plateau potentials that were associated with slow spikes only (Fig. 8A, TEA + CCh + TTX). Moreover, the duration of the plateau potentials did not appear to be significantly changed by TTX, although in two of five neurons we could observe a prolongation. These plateau potentials were abolished by atropine (Fig. 8A, TEA + CCh + TEA + atropine).

In six additional subicular cells treated with $\text{K}^{+}$ channel blockers (TEA or TEA + 4AP, 4 and 2 neurons, respectively) and CCh, we tested the effects induced by $\text{Na}^{+}$ replacement on the depolarizing plateau potentials. Application of choline-containing ACSF abolished the depolarizing pulse-triggered plateau potentials in all experiments (Fig. 8B). A quantitative

---

**FIG. 4.** Voltage dependence of the depolarizing plateau potentials recorded during application of CCh (100 $\mu$M). A: small changes in membrane potential (i.e., $\pm 2$ mV from an RMP = $-68$ mV) increase or decrease the duration of the plateau potentials induced by pulses of depolarizing current of fixed intensity and duration (10 ms, $+0.5$ nA, stimulation interval = 120 s). B: injection of a brief (duration = $20$ ms) hyperpolarizing pulse immediately after the initial depolarizing command makes the plateau potential decrease in duration and eventually disappear. Note the AHP that occurs at the end of the plateau potentials (* in middle sample). C: injection of hyperpolarizing current pulses during the full-blown plateau potential aborts it. Note that in this case the hyperpolarizing command has a triangular shape and its termination gives origin to recurrent action potential bursts.

374 ms, $n = 6$; data obtained 6–14 min after start of the recording) that followed the action potential burst induced by an intracellular depolarizing current pulse (Fig. 7B, +8-min sample). Later the amplitude and the duration of these depolarizing plateau potentials decreased over time, and after 25–40 min of continuous recording, only a small depolarizing plateau potentials similar to the depolarizing afterpotential seen with low CCh concentrations were elicited (maximal duration = $134 \pm 42$ ms, $n = 5$; Fig. 7B, +20- and +35-min samples). At this time, subicular neurons continued to respond to depolarizing current pulses with action potential bursts.

$\text{Na}^{+}$-mediated conductances and plateau potentials

Action potential bursts generated by subicular neurons in control ACSF are abolished by TTX (Mattia et al. 1993, 1997a,b). These burst responses were also blocked by TTX in the presence of CCh ($n = 7$, Fig. 1A). Such an effect was characterized by the absence of regenerative events even during large-amplitude depolarizing commands and accompanied by disappearance of both depolarizing afterpotentials and full-blown plateau potentials ($n = 4$; not illustrated). We reasoned that the TTX-induced depression of plateau potentials may reflect the inability of subicular bursting cells to produce high-threshold $\text{Ca}^{2+}$ spikes in the absence of functional voltage-gated $\text{Na}^{+}$ channels (Mattia et al. 1997a). Indeed, high-threshold $\text{Ca}^{2+}$ spikes can be recorded in subicular bursting neurons when TTX is applied concomitantly with the $\text{K}^{+}$ channel blocker TEA (Mattia et al. 1997a,b). Hence, we analyzed the involvement of $\text{Na}^{+}$-mediated electrogenesis in the occurrence of CCh-induced plateau potentials by pretreating subicular neurons with extracellular application of TEA (10 mM).
summary of the data of the plateau potentials recorded in the experiments performed with TTX and Na\(^+\) replacement is provided in Fig. 8C.

**DISCUSSION**

Our study shows that CCh modifies the intrinsic excitability of bursting neurons in the rat subiculum and discloses depolarizing plateau potentials that are triggered by brief pulses of intracellular depolarizing current. These effects are seen in the presence of ionotropic excitatory amino acid receptor antagonists as well as during further blockade of type A and type B GABA receptors and result from the activation of muscarinic receptors. Accordingly, the changes in intrinsic excitability induced by CCh, along with the plateau potentials, are blocked by atropine but are not influenced by either nicotinic or glutamate metabotropic receptor antagonists. Finally we have provided evidence suggesting the involvement of a nonselective cationic conductance for the generation of CCh-induced plateau potentials.

**Carbachol effects on the intrinsic excitability and burst responses**

Under control conditions, most subicular neurons generate bursts of action potentials that are followed by burst-AHPs (Mason 1993; Mattia et al. 1993, 1997a,b; Stewart and Wong 1993; Taube 1993). In this study, we analyzed the effects exerted by CCh on the intrinsic excitability of this cell subtype because burst firing modality is recorded in the subiculum of freely moving animals during learning tasks (Barnes et al. 1990; Sharp and Green 1994) and because cholinergic mechanisms do influence cognitive processes (Dunnett and Fibiger 1993; Huerta and Lisman 1993; Shen et al. 1994; Winkler et al. 1995). Therefore we assumed that subicular bursting neurons could represent a suitable target for the cholinergic modulation of limbic functions.

Our findings indicate that CCh causes a small, yet consistent, steady depolarization of the membrane potential, an augmentation of the membrane input resistance, and a pronounced reduction, or blockade, of the AHPs that follow action potential bursts (i.e., burst-AHPs) as well as single action potential firing (i.e., fast AHPs). We also have discovered that all the effects induced by CCh are antagonized by atropine, and thus they are due to muscarinic receptor activation. Muscarinic receptor-mediated excitatory effects have been reported in several cortical structures including the hippocampus proper and the entorhinal cortex (Andrade 1991; Benardo and Prince 1982; Constaniti et al. 1993; Engisch et al. 1996; Fraser and MacVicar 1996; Haj-Dahmane and Andrade 1998; Halliwell and Adams 1982; Klink and Alonso 1997a,b; Madison et al. 1987; Schwindt et al. 1988). It also has been established that these actions are associated with decrease of several K\(^+\) currents, including those that are dependent on Ca\(^{2+}\) activation (cf. Krnjević 1993). Indeed, previous findings indicate that in subicular bursting cells both burst-AHPs and fast AHPs depend on Ca\(^{2+}\) entry (Mattia et al. 1993, 1997a,b).
In subicular neurons the blockade of the burst-AHP by CCh results in the occurrence of action potential firing that follows the initial burst and "replaces" the period occupied by the burst-AHP under control conditions. As a result, the overall effect of muscarinic activation in these cells is an increase of action potential discharge during depolarizing commands. In addition, termination of the depolarizing pulse is followed by a slow afterdepolarization that eventually can become a full-blown depolarizing plateau potential (see following text). It also should be emphasized that even large concentrations of CCh (e.g., 50 mM) did not cause any measurable change in the ability of subicular neurons to fire action potential bursts. Such a response is largely due to the activation of a TTX-sensitive Na\(^+\) conductance (Mattia et al. 1993, 1997a,b). CCh reduces voltage-gated Na\(^+\) currents in isolated hippocampal neurons (Cantrell et al. 1996) and also decreases action potential amplitude and rate of rise in entorhinal cortex layer II neurons (Klink and Alonso 1997a). Action potential bursts may play a special role in synaptic plasticity and information processing (Lisman 1997). Hence the resistance of action potential burst firing to CCh may represent an important characteristic of subicular neurons involved in cognitive functions during cholinergic activation.

**Carbachol-induced depolarizing plateau potentials**

CCh application also makes subicular bursting cells generate depolarizing plateau potentials in response to brief pulses of depolarizing current that are only capable of eliciting bursts of action potentials under control conditions. We have shown that these plateau potentials are voltage-dependent (cf. Fraser and MacVicar 1996). First, their magnitude depended on the intensity and/or duration of the triggering depolarizing pulse and was influenced by injecting hyperpolarizing commands immediately after the depolarizing pulse. Second, the plateau potential size decreased or increased when the membrane potential was hyperpolarized or depolarized, respectively, by a few millivolts. Third, injection of hyperpolarizing current during the plateau potential could abort it. Moreover, plateau potential occurrence depended on the ability of the subicular neurons to generate an initial burst of fast action potentials (see following text). This evidence suggests that both plateau potential occurrence and size can be modulate by depolarizing and hyperpolarizing inputs. Under normal conditions (i.e., in the absence of excitatory and inhibitory receptor antigons), these inputs represent excitatory and inhibitory postsynaptic potentials.

We also have documented that plateau potential generation is characterized by a refractory period of several tens of seconds. The mechanisms underlying this phenomenon are unknown. However, it may be speculated that such refractoriness may prevent excessive neuronal depolarization leading to both activation of high-voltage Ca\(^{2+}\) conductances and release of Ca\(^{2+}\) from intracellular stores. Sustained elevation of [Ca\(^{2+}\)],
has been implicated in cellular damage. Indeed, during continuous application of CCh the subicular bursting cells studied here did not show any measurable sign of functional damage (such as changes in input resistance or action potential amplitude/duration) and were capable of generating plateau potentials of similar magnitude over prolonged periods of time (≤3 h).

The CCh-induced plateau potentials are caused in subicular bursting neurons by the activation of muscarinic receptors. Atropine application abolished them, whereas the nicotinic receptor antagonist d-tubocurarine failed in doing so. It should be noted that CCh-induced plateau potentials occurred in the presence of ionotropic excitatory amino acid receptor antagonists and were not influenced by further application of GABA_A and GABA_B receptor antagonists. This pharmacological evidence reinforces the conclusion that CCh-induced plateau potentials are intrinsic neuronal events not contributed by excitatory or inhibitory synaptic conductances. We also ruled out the possible involvement of metabotropic glutamate receptors that may be activated by excitatory amino acid transmitters released from presynaptic terminals during CCh application. Metabotropic glutamate receptor activation induces and/or potentiates slow afterdepolarizations in cortical neurons (Constanti and Libri 1992; Greene et al. 1994) and causes the appearance of prolonged bursts of action potentials (Bianchi and Wong 1995). How-

---

**FIG. 7.** Ca\(^{2+}\) dependence of the plateau potentials induced by CCh. A: extracellular application of Co\(^{2+}\) (2 mM) greatly reduces the depolarizing plateau potentials, whereas the initial action potential bursts still are elicited by brief current pulses. Note that the action potential bursts recorded in CCh + Co\(^{2+}\) are followed by a depolarizing afterpotential (↓); note also that the low-intensity, intracellular pulse that is unable to trigger action potential burst during CCh application, does so after Co\(^{2+}\) addition. *B*: intracellular recordings obtained with an electrode filled with K-acetate + bis(2-aminophenoxy)ethane-N,N',N''-tetraacetic acid (BAPTA) in the presence of CCh. An action potential burst-depolarizing plateau potential sequence is triggered by an intracellular depolarizing current pulse shortly after the impalement (8-min sample); later (+20-min sample) the plateau potential is greatly reduced. Note that 35 min after the impalement only a small depolarizing plateau potential can be elicited by an intracellular current pulses even when the intensity is more than doubled.
ever, the metabotropic receptor antagonist MCPG did not influence CCh-induced plateau potential duration or the ability of depolarizing pulses to induce them. Depolarizing plateau potentials similar to those reported here have been recorded in the presence of CCh from CA1 pyramidal cells (Fraser and MacVicar 1996) and entorhinal cortex layer II neurons (Klink and Alonso 1997a,b). Slow poststimulus depolarizations also occur in CA3 pyramidal cells (Caeser et al. 1993), olfactory cortex neurons (Constanti et al. 1993), and neocortical neurons (Andrade 1993; Schwindt et al. 1988) during cholinergic activation. In all these studies, the plateau potentials or the slow poststimulus depolarizations were dependent on muscarinic receptor activation.

### Ionic mechanisms underlying the plateau potentials

Plateau potential generation in subicular bursting neurons depends on the occurrence of an initial burst of fast action potential that is mainly caused by a voltage-gated Na⁺ mechanism (Mattia et al. 1993, 1997a,b). In line with this conclusion, CCh-induced plateau potentials, along with the initial action potential bursts, were abolished by the voltage-gated Na⁺ channel blocker TTX. This effect, however, does not reflect an action of TTX on the depolarizing conductances underlying the plateau potential per se but rather the disappearance of the initial action potential burst exerted by TTX. Accordingly, plateau potentials could be triggered during con-
comitant application of CCh, TTX, and TEA (or TEA + 4AP). TEA is a K\(^+\) channel blocker that makes subicular cells generate presumptive Ca\(^{2+}\) spikes in response to depolarizing pulses even when voltage-gated Na\(^+\) conductances are abolished (Mattia et al. 1997a). This evidence suggests that plateau potential generation in subicular cells depends on the ability of depolarizing pulses to produce regenerative events that in turn depolarize sites remote to the soma such as the dendrites.

Previous studies from different cortical preparations have demonstrated the insensitivity of muscarinic plateau potentials to TTX (Costanti et al. 1993; Fraser and McVicar 1996; Klink and Alonso 1997b). Moreover, the sole application K\(^+\) channel blockers was not sufficient to elicit plateau potentials in our preparation (cf. Fraser and MacVicar 1996), suggesting that a decrease in K\(^+\)-mediated hyperpolarizing conductances is not sufficient to make subicular cells generate prolonged depolarizations such as those seen during the CCh-induced plateau potentials. As reported in other cortical cells (Fraser and MacVicar 1996; see also Constanti et al. 1993), the plateau potentials recorded during application of TEA + CCh were shorter than those seen in medium containing CCh only.

Colino and Halliwell (1993) have reported that activation of muscarinic receptors in the hippocampus potentiates an inward current that causes the time and voltage-dependent hyperpolarizing rectification that also is seen in subicular bursting neurons (Mattia et al. 1993, 1997a). However, CCh-induced plateau potentials were not influenced by extracellular application of Cs\(^+\), a procedure that effectively abolished the hyperpolarizing rectification and the sags recorded in subicular cells (cf. Mattia et al. 1993). Hence, the depolarizing plateau potentials described here are not caused by a muscarinic-dependent decrease of I\(_0\). Moreover, the amplitude and/or duration of the plateau potentials did not change over time during recording made with KCl-filled electrodes. Hence, we can exclude the contribution of Cl\(^-\)-mediated conductances to the CCh-induced plateau potentials recorded in the subiculum (Constanti et al. 1993; Fraser and MacVicar 1996).

We also have demonstrated that CCh-induced plateau potentials are reduced markedly by applying ACSF where most Na\(^+\) was replaced with choline. This pharmacological procedure was performed during application of TEA, because under control conditions (i.e., in medium containing CCh) Na\(^+\) replacement lead to disappearance of the initial burst that is instrumental for plateau potential occurrence. TTX-sensitive fast transients disappeared during choline application, but subicular cells were still able to generate an initial large amplitude slow spike response that was abolished by Co\(^{2+}\) and thus represented a presumptive high-threshold Ca\(^{2+}\)-mediated regenerative event. However, in spite of this initial depolarizing response, subicular neurons were unable to generate plateau potentials during application of low Na\(^+\) medium. Hence, TTX-insensitive influx of Na\(^+\), along with other cations such as Ca\(^{2+}\), is instrumental for the generation of muscarinic plateau potentials in the subiculum.

**Ca\(^{2+}\) dependence of the plateau potentials**

We also have documented that CCh-induced plateau potentials depend on Ca\(^{2+}\) entry because they were reduced markedly and abolished by voltage-gated Ca\(^{2+}\) channel blockers. Moreover they disappeared over time during intracellular recordings performed with electrodes containing the Ca\(^{2+}\) chelator BAPTA. Therefore in addition to voltage-gated Ca\(^{2+}\) conductances, the generation of plateau potentials induced by CCh is contingent on the release of Ca\(^{2+}\) from intracellular stores. It has been shown that muscarinic receptors (in particular the m1 subtype) activates an intracellular messenger cascade that leads to Ca\(^{2+}\) mobilization from intracellular stores (Berridge and Irvine 1989; McKinney 1993). Therefore our findings suggest that Ca\(^{2+}\) plays a role in triggering the plateau potentials and also contribute to the depolarizing plateau potential itself.

Overall, the evidence presented here suggests that the plateau potentials generated by subicular cells reflect an intrinsic voltage-gated mechanism that is caused by a Ca\(^{2+}\)-activated nonselective cationic conductance. This type of conductance is present in a broad range of cell types, and it has been implicated in the generation of plateau potentials (or prolonged afterdepolarizations) in neurons located in other limbic structures (Caesar et al 1993; Fraser and MacVicar 1996; Klink and Alonso 1997a,b) as well as in the neocortex (Andrade 1991; Schwindt et al. 1988). We are inclined to propose that during muscarinic activation, depolarizing commands delivered through the recording electrode presumably located at the soma cause a cascade of events that are initiated by 1) generation of a Na\(^+\)-mediated action potential burst leading to, 2) activation of high-threshold Ca\(^{2+}\) channels, presumably located on the dendrites, and 3) release of Ca\(^{2+}\) from intracellular stores. In turn the latter two Ca\(^{2+}\)-mediated events lead to the activation of a nonselective cationic conductance. Such a process is facilitated by the depression of several K\(^+\)-mediated hyperpolarizing mechanism exerted by CCh.

**Conclusions**

We have shown here that CCh induces profound changes in the function of subicular bursting neurons by blocking AHPs, increasing their intrinsic excitability, and promoting the appearance of depolarizing plateau potentials. All these effects are caused by the activation of muscarinic receptors. Acetylcholine is involved in neuronal plasticity, whereas the subiculum is implicated in cognitive functions such as spatial learning (Barnes et al. 1990; Sharp and Green 1994). Hence our result are relevant for understanding the mechanisms underlying physiological processes such as learning and memory as well as pathological conditions. Indeed the subiculum along with the entorhinal cortex presents the most severe cell loss in individuals with Alzheimer’s disease (Miller et al. 1987; Samuel et al. 1987).

The strategic position occupied by the subiculum within the hippocampal-entorhinal cortex loop also suggests that the changes in excitability induced by CCh may contribute to epileptic synchronization. Previous studies have shown that cholinergic agents induce epileptiform discharges that are caused mainly by muscarinic receptor activation (Dickson and Alonso 1997; Nagao et al. 1996; Turski et al. 1989). In particular, the depolarizing plateau potentials generated by subicular neurons described here, along with those generated by other cortical neurons, may play a central role in limbic seizure generation and thus in temporal lobe epilepsy. In line with this view, recent findings indicate that the antiepileptic drug topiramate can reduce the plateau potentials induced by CCh in the
rat subiculum (Avoli et al. 1998). Hence muscarinic excitation may represent a good, as yet unexplored, target for antiepileptic drug action.

We are grateful to Dr. K. Kmiecik for critical comments on this manuscript. We also thank R. Motalli and T. Papadopoulos for research and secretarial assistance.

This work was supported by grants from the Medical Research Council of Canada (MT-8109), the Savoy Foundation, the Hospital for Sick Children Foundation (XG-93056), and the Quebec Heart and Stroke Foundation.

Address for reprint requests: M. Avoli, 3801 University St., Montreal, QC H3A 2B4, Canada.

Received 3 May 1999; accepted in final form 14 July 1999.