Characterization of Carbachol-Induced Rhythmic Bursting Discharges in Neurons From Guinea Pig Lateral Septum Slices

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Carette, Bernard. Characterization of carbachol-induced rhythmic bursting discharges in neurons from guinea pig lateral septum slices. J. Neurophysiol. 80: 1042–1055, 1998. A brain slice preparation from guinea pigs and intracellular recording techniques were used to examine the effects of carbachol application on the three classes (A, B, and C) of neurons (n = 68, 40 of class A, 12 of class B, 16 of class C) within the mediolateral part of the lateral septum (LSml). Bath application of carbachol elicited a sustained depolarization associated with an increase in membrane input resistance, action-potential firing and triggered rhythmic bursting discharges in 59% of recorded neurons. According to the configuration of these bursts, LSml neurons were classified into type I, II, and III neurons with reference to their response to carbachol. The frequency of spontaneous bursts was increased by depolarization caused by applied DC current in the three types of neurons. Bursts in type II and III neurons were voltage and dose dependent. These dependences were responsible for a continuum of variation in carbachol responses in these two types of neurons. As the neuron depolarized in the presence of carbachol, spontaneous action potentials increased in frequency and slow afterdepolarizing potentials (sADPs) appeared and preceded the occurrence of the first burst. These sADPs from adjacent action potentials appeared to progressively increase to initiate a burst. In the presence of carbachol, sADPs and bursts were also observable after action potentials evoked by depolarizing current pulses at the resting membrane potential (RMP) in LSml neurons. Evoked sADPs and bursts were associated with an apparent increase in input conductance. Application of low Na+ medium blocked both the sADP and bursts. Application of zero Ca2+ medium either 1) blocked completely the generation of sADPs and bursts (n = 16), or 2) did not block bursts (n = 14). Evoked sADPs and bursts were blocked by tetraethylammonium but were resistant to external Cs+. The results indicate that the activation of cholinoergic receptors does not differentially affect the three classes of LSml neurons. The responses to carbachol in type II and III neurons form a continuum of variation, whereas these of type I neurons constitute a discrete entity. The selective cholinoergic induction of a sADP, and the progressive activation of these sADPs in LSml neurons are thought to be responsible for the onset of the three types of rhythmic bursting discharges. We propose that sADPs and bursts induced by carbachol are generated by a cationic conductance largely permeable to Na+. In a subpopulation of LSml neurons (n = 16), the bursts are dependent on the presence of Ca2+ in the medium.

INTRODUCTION

Recently, we reported that selective activation of muscarinic receptors elicits three types of rhythmic bursting discharges in guinea pig mediolateral part of the lateral septum (LSml) neurons (Carette 1997). These discharges were different according to the neurons, depending on the duration, the amplitude, and the waveform of the bursts. Thus three types (I, II, and III) of LSml neurons have been distinguished according to their response to carbachol. These different bursting discharges were not blocked by a medium that contained tetrodotoxin (TTX), and their frequency was dependent on the membrane potential. Therefore it was concluded that LSml neurons present conditional bursting properties revealed by application of carbachol.

On the other hand, the intrinsic membrane properties of LSml neurons were previously investigated in vitro (Carette et al. 1992). On the basis of responses to depolarizing current pulses in control conditions and in the presence of TTX, we have characterized three classes (A, B, and C) of LSml neurons. Neurons of classes A and C represent the large majority and exhibit similar morphologies (Carette et al. 1992; Dourelant et al. 1994).

In this paper, we have attempted to answer several questions. 1) Can the occurrence of different types of bursts induced by carbachol in LSml neurons be in correlation with the three classes of these neurons? 2) Are types I, II, and III discrete entities, or do they represent a continuum of response to carbachol? 3) What properties and ionic mechanisms are involved in the carbachol-induced rhythmic bursting activities?

METHODS

Experiments were performed on transversely cut slices (400 μm thick) of lateral septum, prepared from female guinea pigs (250–300 g). Slices were maintained at 32°C in an interface chamber and perfused continuously (2 ml/min) with solution of the following composition (in mM): 117 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 NaH2PO4, 25 NaHCO3, 1.2 MgCl2, and 10 glucose, bubbled with 95% O2-5% CO2 (pH 7.4). Low Na+ medium and zero Ca2+ medium were made by replacement of 117 mM NaCl with 117 mM tris-(hydroxymethyl)-aminomethane chloride (TrisCl) and by replacement of CaCl2 with MgCl2 on an equimolar basis, respectively. Cobalt (Co2+) or nickel (Ni2+) were substituted at equimolar concentrations for Ca2+. For this purpose, CaCl2 was replaced with 2.5 mM CoCl2 or NiCl2 when preparing the medium, whereas NaH2PO4 was omitted to avoid precipitation. The drugs used in this study were carbachol, TTX, tetraethylammonium (TEA), and cesium (Cs+; all purchased from Sigma). All drugs were administered in the perfusate dissolved at known concentrations, during at least 3 min of application.

Electrophysiological recordings were obtained from neurons in LSml, using intracellular recording techniques as previously described (Carette et al. 1992). Recordings were obtained using electrodes filled with 3 M KCl (resistance 50–80 MΩ) or with 3 M K acetate (resistance 100–150 MΩ). Intracellular signals were amplified using an Axoclamp-2A amplifier and displayed on a chart recorder (GOULD Model 3400). All signals were stored on tape for later detailed analysis.
Intrinsic membrane properties of LSml neurons

As previously found (Carette et al. 1992), 68 neurons fell into three distinct electrophysiological classes: A, B, and C, whose main characteristics are summarized in Fig. 1. When they were challenged with depolarizing current pulses from rest, the LSml neurons of A and B classes discharged tonically in control conditions (Fig. 1A). In the presence of TTX (1 μM), class A neurons (n = 40) were characterized by broad action potentials (Fig. 1B) in response to a depolarizing current pulse. These broad action potentials were blocked by 2 mM CoCl_2 (not shown). Class B neurons (n = 12) had no action potential under TTX (Fig. 1C). Action potentials were recorded only by addition of TEA (1–2 mM) to TTX (not shown) and were also blocked by CoCl_2 (not shown). The firing properties of the class C neurons (n = 16; Fig. 1D) could be readily distinguished in control conditions from those of two other classes, by their tendency to show small spikes (blocked by TTX; not shown) followed by larger spikes (blocked by CoCl_2; not shown) in response to depolarizing current pulses. Under control conditions, the three classes of LSml neurons discharged in a single-spike mode in the presence of a steady depolarizing current (not shown).

Responses to cholinergic agonists

As previously reported (Carette 1997), LSml neurons responded to bath application of carbachol (20 μM) by a membrane depolarization associated with an increase in membrane resistance and in tonic firing rate (not shown). The depolarization by carbachol was observed in the three classes of neurons: 24 for class A, 6 for class B, and 10 for class C. Then the firing pattern changed from single spikes to repetitive bursts, i.e., clusters of four or more closely spaced action potentials riding on a distinct depolarizing envelope. Individual bursts varied in waveform, amplitude, and duration according to the neurons (Fig. 2). We classified these LSml neurons into three types based on their bursting behavior in response to carbachol. In type I neurons (n = 13), the burst consisted of 4–6 spikes rising from a slow membrane depolarization (Fig. 2B, left trace). In type II neurons (n = 18), bursts elicited by carbachol consisted of a depolarizing drive termed the plateau potential, which sustained action potentials whose number was higher than 6 (Fig. 2A, bottom trace). In type III neurons (n = 36), bursts consisted of a plateau depolarization of large amplitude with spikes that quickly inactivated. Membrane potential typically remained elevated for several hundred milliseconds following spike inactivation, resulting in a temporary cessation in firing (Fig. 2C, left trace). These three types of neurons were observed in each class of LSml neurons (Table 1). The three types of bursts in class C neurons are shown in Fig. 1E.

Other neurons either showed no depolarization (n = 22) or were hyperpolarized by carbachol (n = 5; not shown). However, in all these neurons (n = 27), additional depolarization by intracellular injection of positive DC readily elicited rhythmic bursting discharges of type I, II, and III. Table
TABLE 1. Association between the effects of CCh on membrane potential and the types of rhythmic bursting discharges induced by CCh in the three classes of LSml neurons

<table>
<thead>
<tr>
<th>Class</th>
<th>Type I Burst</th>
<th>Type II Burst</th>
<th>Type III Burst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class A neuron</td>
<td>39</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Depolarization</td>
<td>2</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Hyperpolarization</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Without effect on the RMP</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Class B neuron</td>
<td>12</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Depolarization</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Hyperpolarization</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Without effect on the RMP</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Class C neuron</td>
<td>16</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Depolarization</td>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Hyperpolarization</td>
<td>2</td>
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<td>3</td>
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*n* is number of neurons. Total number of LSml neurons used was 67. CCh, carbachol; LSml, mediolateral part of the lateral septum; RMP, resting membrane potential.

1 shows the association between the effects of carbachol on membrane potential and the types of rhythmic bursting discharges induced by carbachol into the three classes of LSml neurons. One class A neuron was unresponsive to carbachol, whereas no class C neurons were hyperpolarized by carbachol.

Voltage dependence of carbachol-induced bursts

To determine whether the three response profiles recorded from LSml neurons were really different among the three types of neurons, positive currents of different amplitudes were continuously applied during the steady-state phase of carbachol-induced responses for each type (6 of type I, 8 of type II, and 19 of type III).

The recordings presented in Fig. 2A show that both types of responses could be recorded from individual neurons, and that the character expressed was determined by the prevailing membrane potential. In the absence of current injection, this neuron (Fig. 2A) showed a rhythmic bursting discharge [resting membrane potential (RMP): −64 mV]; middle traces: in presence of a depolarizing current injection (+50 and +150 pA), the frequency and duration of the bursts increased; top trace: continuous injection of +200 pA depolarizing current transformed the type II burst into a type III burst, whereas the frequency of the bursts decreased. B, left trace: response of type I neuron (RMP: −58 mV) in absence of current injection; right trace: with +200 pA, the waveform and the duration of the burst were unchanged, whereas an inactivation of action potentials was observed. C, left trace: response of type III neuron (RMP: −65 mV) in absence of current injection; right trace: with +200 pA, the duration of the burst decreased, whereas its time course was changed.

Dose dependence of carbachol-induced bursts

To determine whether the three response profiles recorded from LSml neurons were really different among the three types of neurons, carbachol was perfused onto a same neuron of each type (n = 3 for type I, n = 3 for type II, and n = 5 for type III), in concentrations ranging from 5 to 50 μM. In type I neurons (Fig. 3A), the burst did not change whatever the concentration of carbachol (5–50 μM). In Fig. 3B, at 5, 10, and 20 μM carbachol, the neuron exhibited type II
Mechanisms of rhythmic bursting discharges

spontaneous burst firing. As previously reported (Carette 1997), LSml neurons in the presence of carbachol displayed a depolarization and an increase of firing rate, the discharge shifting then from single spiking to bursting. The time course of this transformation for type I and II neurons is shown in Fig. 4. With time from onset of carbachol superfusion, marked changes occurred in the spike afterpotential: the action potential of LSml neurons developed a slow afterdepolarizing potential (sADP). The size of the sADP increased with time, attaining spike threshold, and this resulted in a spike doublet (Fig. 4, A and C) or a spike burst (Fig. 4, A–C). Thus the occurrence of the first burst in type I and II neurons was preceded by the appearance of a sADP. In class C neurons, the sADP occurred after a Ca$^{2+}$ spike (Fig. 4C). These observations suggest that the sADP is a determining factor of the bursting onset in type I, II, and III LSml neurons in the presence of carbachol.

EAKED BURST FIRING. Muscarinic-mediated sADP after evoked action potentials. In the presence of carbachol, sADPs were also associated with action potentials evoked by intracellular current pulses in LSml neurons whose membrane potential was clamped back to the control value (RMP) by continuous injection of current. The application of a single (4 ms) depolarizing current pulse to LSml neurons at the RMP evoked an action potential with a slow afterhyperpolarization (sAHP; Fig. 5A) or without an afterpotential (Fig. 5B) in control. This sAHP was apamin sensitive (Carette 1994). In the presence of carbachol, a sADP followed a single action potential evoked by the same pulse, in neurons of type I (n = 3), type II (n = 8), and III (n = 39; Fig. 5, A and B). The peak sADP amplitude was 8 ± 3.4 mV (mean ± SD, n = 28) and duration was 137.1 ± 45.6 ms (n = 28). In the remaining neurons of type I (n = 23), II (n = 11), and III (n = 20), more than one action potential was required to evoke a sADP (Fig. 5, C and D).

Progressive activation of SADPs and triggering of plateau potential. The sADP enhanced temporally in amplitude when the number of action potentials was increased by the depolarizing current pulses (Fig. 5, C and D). In some neurons (n = 26), the peak amplitude of this sADP attained a maximum value whatever the intensity and width of pulses and therefore the number of action potentials (Fig. 5C). This response was similar in waveform, amplitude, and duration to the carbachol-induced spontaneous burst obtained at the membrane potential for spike initiation in type I neurons. However, in a majority of neurons (n = 78), the response to depolarizing current pulses, in the presence of carbachol, consisted of two phases: successive sADPs enhanced in amplitude and, when the depolarization reached a certain voltage threshold, the membrane potential jumped to form an underlying depolarizing drive, i.e., the plateau potential that sustained repetitive firing (Fig. 5D) or showed an inactivation of spikes (not shown). This plateau potential occurred typically in all-or-none fashion. These two responses were similar in waveform to carbachol-induced bursts obtained in type II and III neurons, respectively. Sometimes, the sADP after one action potential was sufficiently large to elicit a plateau potential in type II and III neurons (not shown).

We have also tested the progressive activation of sADPs by applying short (4 ms) depolarizing current pulses repetitively at different interpulse intervals (n = 40). In type I neurons, the repeated stimulation (4-ms pulses, frequency: 2.5–10 Hz) in the presence of carbachol resulted in the cumulative activation of the sADP but allowed the induction of a short burst (Fig. 6A). Different stimulation frequencies...
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FIG. 4. Appearance of spontaneous action potentials, slow afterdepolarizing potentials (sADPs), and bursts in LSml neurons in the presence of CCh. A: as this type I neuron depolarized under CCh (left trace), the 1st spontaneous activity to appear was a regular firing of action potentials. The occurrence of a sADP was characterized by the presence of spontaneous spike doublets (middle trace in 1). After 4–6 min in the test solution, bursts of action potentials (right trace, arrowhead) in addition to the single action potentials appeared. B: this neuron of type II showed a regular firing of action potentials in the control solution (top left trace). In the presence of CCh (top right trace), an increase of firing frequency was observed, followed by the appearance of 1st sADPs and bursts. Bottom traces: expanded voltage traces were obtained at the different times shown in the continuous record (top right trace) to illustrate changes in activity. Bottom middle trace: appearance of 1st sADPs (arrows). Voltage calibration in B also applies in A and C. C, left trace: spontaneous action potentials in control solution, in this class C neuron. Under CCh, appearance of a sADP, followed by that of a spike doublet (middle traces) and a type I burst (right trace).

should be more or less effective to allow for the activation of sADPs and for triggering of plateau potentials in type II (Fig. 6B) and type III neurons (not shown). Thus, in Fig. 6B (left trace), at a frequency of 3.3 Hz, this neuron exhibited a time-dependent change in spike afterpotentials with the appearance of a sADP, but a sAHP still followed the sADP and a plateau potential was not observed. With an interpulse interval of 200 ms, there was a gradual increase of the sADP with disappearance of the sAHP, and a plateau potential appeared (Fig. 6B, right trace).

In our study the appearance of a sADP in the presence of carbachol might simply be a consequence of blockade of the sAHP. However, our results argue against this idea. First, in neurons without a sAHP at RMP in control conditions, carbachol induced a sADP (Fig. 5B); second, in neurons with a sAHP in control conditions, this sAHP was still present under carbachol after one action potential, whereas the appearance of a sADP needed several action potentials (Fig. 5C). Moreover, in the example of Fig. 6B, whereas a sADP was induced by carbachol from successive spikes, a sAHP was still present after this sADP (left trace). The right trace of Fig. 6B shows that the disappearance of the sAHP was concomitant of a more important activation of sADPs by an increased frequency of action potentials. The fact that we simultaneously recorded a sADP and a sAHP in the same neuron under carbachol excluded the idea that the sADP was the consequence of blockade of a sAHP. These findings point to the conclusion that the sADP was actually induced rather than unmasked by muscarinic agonists.

Voltage and dose dependence of sADPs

The sADPs were modulated by membrane potential. They were maximal in amplitude at RMP, and reduced or suppressed by hyperpolarization (Fig. 7A). At a depolarizing level, the sADP triggered a burst (not shown).

In several neurons (n = 6) for which a sADP was induced by carbachol following a single spike, we varied the concentration of the cholinergic agonist to investigate whether sADP amplitude was graded with concentration. Figure 7B shows how sADP amplitude increased after progressively larger doses (10 and 40 μM) of carbachol. At 5 μM carbachol, a sADP did not appear after a single spike but appeared after three spikes (Fig. 7B, right trace).
Modulation of evoked bursts by membrane potential

The configuration of bursts induced by carbachol and evoked by depolarizing current pulses was also sensitive to changes in membrane potential caused by applied DC current. In type I neurons, two bursts were evoked at a depolarizing level, whereas at the RMP, one burst was present (Fig. 8A, left and middle traces). At a hyperpolarizing level, the burst evoked decreased in number of spikes (Fig. 8A, right trace). As illustrated in Fig. 8B, hyperpolarizing the type III neuron decreased the size of the bursts (amplitude and duration), whereas the spikes were not inactivated (left and middle traces). At a same hyperpolarizing level, a sADP appeared after a single spike (Fig. 8B, right trace). Thus a complete cessation of carbachol-induced spontaneous bursting by hyperpolarization was obviously a result of reduction of the evoked burst by the same hyperpolarization.

Changes in membrane input resistance ($R_m$) during bursts and sADPs

In type III neurons, it was possible to monitor the $R_m$ changes accompanying the plateau potential because action potentials quickly inactivated. Brief hyperpolarizing current pulses applied before and during the plateau potential indicated a large reduction in $R_m$ during the plateau potential (usually so low that the voltage deflection could not be measured reliably within 200 ms after onset of the plateau potential; not shown). In type III neurons, the amplitude of a typical plateau potential decayed by 4–10 mV over a time course of 800 ms to 4 s, then 30–40 mV over a time course of 100–400 ms (Fig. 2C, left trace). This decay was associated with an increase in $R_m$ as revealed by the increasing amplitude of voltage responses to the series of current pulses delivered during the plateau potential (not shown). Thus the plateau potential represents a state of high membrane conductance, and a progressive decrease in conductance is
FIG. 6. In the presence of CCh, progressive activation of sADPs and triggering of plateau potential in 2 LSml neurons. Depolarizing current pulses (4 ms) evoking a single action potential were delivered at various frequencies. A: in this type I neuron (RMP: $-61$ mV), a sADP was absent after the 1st action potential, in the presence of CCh (bottom left trace). A progressive activation of sADPs was observed at different frequencies (2.5, 3.3, and 10 Hz) of stimulation. However, at 2.5 Hz, the amplitude of sADP was insufficient to trigger another action potential (top left trace). With an increase of frequency stimulation (3.3 Hz), the amplitude of sADPs increased and a burst appeared after the 11th pulse (top right trace). At 10 Hz (bottom right trace), the burst appeared more quickly (5th pulse). Bottom left traces: superimposed records of action potentials and sADPs in response to the 1st, 10th, and 25th pulses at 2.5 and 3.3 Hz. The sADP had the same amplitude in response to the 10th and 25th pulses at 2.5 Hz. Calibrations in A (bottom right trace) apply also in B except for time. B: in this type II neuron (RMP: $-65$ mV), a sAHP (left trace, arrow) was present after the 1st action potential in the presence of CCh. Low-frequency stimulation (3.3 Hz) evoked the appearance of a sADP (arrowhead) but not that of a plateau potential, whereas the sAHP was still present (left trace). With an increase of frequency stimulation (5 Hz), sADPs increased in amplitude, and the threshold for initiation of a plateau potential was reached (right trace).

associated with the decay leading to plateau potential repolarization.

To measure the $R_m$ change during the sADP, we delivered brief hyperpolarizing current pulses near the sADP peak and in the absence of a sADP. The voltage deflections markedly decreased during the sADP, indicating a considerable decrease in $R_m$ (not shown). Depolarization of the membrane to a level similar to that of the sADP peak, in the absence of a preceding spike, did not cause a decrease in $R_m$ (not shown). It is suggested that the sADP is associated with activation of inward currents rather than blockade of outward currents.

**Effects of TTX on bursts and sADPs**

As previously reported (Carette 1997), the three types of rhythmic bursting activities induced by carbachol persisted under TTX. In the present study, a similar observation was obtained in each class of LSml neurons ($n = 39$ for class A, $n = 12$ for class B, and $n = 9$ for class C). As shown

FIG. 7. Voltage and dose dependence of evoked sADPs in the presence of CCh. A: an action potential was evoked by a 4-ms depolarizing current pulse. In the presence of CCh, the sADP was maximal in amplitude at RMP ($-63$ mV; left trace) and was reduced (middle traces), then suppressed (right trace) by a progressive hyperpolarization. B, left trace: response to a 4-ms depolarizing current pulse in control conditions. In the presence of 5 μM CCh, no sADP was observed after 1 action potential. In the same neuron, a sADP appeared with 10 μM CCh and increased in amplitude with 40 μM CCh after 1 action potential. In the presence of 5 μM CCh, a sADP appeared after 3 action potentials (right trace).
in Fig. 9A for a class A neuron, in the presence of TTX, the carbachol-induced plateau potential was still observed and was able to sustain a train of high-threshold Ca$^{2+}$ spikes (Fig. 9A, right trace). For comparison, the response under carbachol was shown with the left trace. As shown in Fig. 9B for a class B neuron treated with TTX and carbachol, elimination of Na$^+$ spikes by TTX did not reveal Ca$^{2+}$ spikes; however, the plateau potential induced by carbachol could be activated by steady depolarization alone, independent of an action potential (Fig. 9B, right trace). For comparison, the response under carbachol was shown with the left trace. As shown in Fig. 9C for a type I neuron of class A, no sADP followed the Ca$^{2+}$ spikes in the presence of TTX (Fig. 9C, middle trace), but a sADP always followed the spikes when carbachol was added (Fig. 9C, right trace). The response of the same neuron under carbachol was shown with the left trace (Fig. 9C).

**Effects of low Na$^+$ medium on bursts and sADPs**

The ions mediating the bursts and sADPs were investigated either by substitution experiments or by including specific channel blockers in the perfusion medium. A role for Na$^+$ was investigated by replacing extracellular Na$^+$ with equimolar Tris. When extracellular Na$^+$ was reduced from 143 to 26 mM, rhythmic bursting discharges induced by carbachol were reversibly abolished in the three types of LSml neurons (n = 19; Fig. 10, A and B). sADPs were compared in medium containing 143 and 26 mM Na$^+$. Both control and test solutions contained 1 μM TTX and 20 μM carbachol. Ca$^{2+}$ spikes (class A neurons) were used to evoke the sADP (Fig. 10C, middle trace). Substitution of 117 mM NaCl by Tris-Cl caused a reversible blockade of sADPs (Fig. 10C, right trace). The response under carbachol was shown with the left trace.

These results clearly indicated that influx of Na$^+$ through TTX-insensitive channels was critical for generation of bursts and sADPs.

**Effects of Ca$^{2+}$-free medium and Ca$^{2+}$ channel blockers on bursts and sADPs**

To investigate the role of Ca$^{2+}$ in the generation of carbachol-induced bursts and sADPs in LSml neurons, we examined the effects of Ca$^{2+}$-free medium and Ca$^{2+}$ channel blockers (2 mM Ni$^{2+}$ or 2 mM Co$^{2+}$) on different types of neurons. Application of these different medium had one of the following effects on the carbachol-induced bursts: 1) reversible blockade of their generation (n = 16, 3 of type I whose 1 class B and 1 class C, 3 of type II whose 1 class A and 10 of type III whose 1 class A and 3 class B; Fig. 11A), 2) absence of blockade of their generation (n = 14, 1 of type I, 4 of type II whose 2 class A and 9 of type III whose 1 class A and 1 class B; Fig. 11B). Bath application of Ca$^{2+}$-free medium or Ni$^{2+}$ blocked sADPs, including in neurons whose bursts were not blocked by these medium (Fig. 11, C and D).

These observations that Ca$^{2+}$-free medium blocked the carbachol-induced bursts and sADPs in about one-half of the LSml neurons (16 of 30 cells) strongly suggest that, at least in certain subpopulation of LSml neurons, Ca$^{2+}$ entry is essential for the burst and sADP generation.

**Effects of K$^+$ channel blockers on bursts and sADPs**

Addition of TEA (1–2 mM) to the medium perfusion reversibly blocked carbachol-induced rhythmic discharges recorded from each type of LSml neurons (n = 6 for type I, n = 8 for type II, and n = 18 for type III; Fig. 12A, type III neuron). In the absence of carbachol, the addition of 1–2 mM TEA to the medium perfusion resulted...
in an increase of Ca$^{2+}$ influx as evidenced by increased spike broadening, and, after cessation of the depolarizing current pulse, a sADP was observed (Fig. 12B, top middle trace). However, in these conditions, neither rhythmic bursting discharges or plateau potentials could be elicited (not shown) in these neurons showing in other respects these events in the presence of carbachol. In Fig. 12B, top right traces, a carbachol-induced plateau potential was easily evoked by a depolarizing current pulse in this LSml neuron, and coapplication of 2 mM TEA blocked this plateau potential. When a sADP was obtained after a single spike in the presence of carbachol, coapplication of TEA also blocked this event (Fig. 12B, bottom right traces).

Another K$^+$ channel blocker, Cs$^+$ (2–5 mM) failed to eliminate bursts and sADPs in the three types of neurons (n = 1 for type I, n = 3 for type II, and n = 5 for type III, not shown). In a majority of LSml neurons, a time- and voltage-dependent anomalous rectification corresponding to a hyperpolarization-activated cation current ($I_h$) blocked by 2 mM Cs$^+$ was present (Fig. 12C, left and middle traces) (Carette et al. 1992). When carbachol induced in type III neurons rhythmic bursting discharges with interburst hyperpolarization, the blockade of the anomalous rectification by Cs$^+$ changed the rhythmicity of bursting discharges (Fig. 12C, right trace).

**DISCUSSION**

The present study indicates that in the three classes of LSml neurons previously defined (Carette et al. 1992), bath application of carbachol induced rhythmic bursting discharges that could emerge either spontaneously, by the direct depolarizing action of carbachol, or on an additional positive current injection. The three types (I, II, and III) of bursting discharges induced by carbachol were observed in each class (A, B, or C) of LSml neurons. Thus the heterogeneity in the response of LSml neurons to applied muscarinic agonists was not due to the existence of these three classes of neurons. On the other hand, a clear relation between the variability in muscarinic responsiveness in neurons of the olfactory cortex and the three morphologically identified neuronal populations in this brain region has been established (Libri et al. 1994).

There is a possibility that the differential effects of carbachol on LSml neurons could be due to the different density of muscarinic receptors in these neurons. Our results show that carbachol produced similar bursts whatever its concentration in type I neurons. Therefore these results suggest that a different density of muscarinic receptors in type I neurons was not at the origin of the differential effect of carbachol in this type of neuron. On the other hand, carbachol produced different bursts both in type II and III neurons according to the concentration. Thus an increase in carbachol concentration in type II neurons shifted the type II response along a continuum toward a type III response. Conversely, a decrease in carbachol concentration in type III neurons shifted the type III response along a continuum toward a type II response. Thus the density of muscarinic receptors appeared different between type II and III neurons. The carbachol-induced bursts in LSml neurons were also voltage dependent, and their frequency was changed in the three types of neurons according to the membrane potential. However, whereas carbachol produced similar bursts whatever the level of membrane potential in type I neurons, it produced different bursts both in type II and III neurons according to the level of membrane potential. Thus, because a type I cannot turn into type II or III whatever the changes in membrane potential or concentrations of carbachol, this type represents a discrete entity, whereas types II and III constitute a continuum of responses to carbachol. Our results support the presence of two interchangeable modes (type II and III) of bursting discharges induced by carbachol from the same LSml neuronal population. The interchange between these two types was dose and voltage sensitive. Therefore, in our study, the type of response to carbachol for each neuron was determined for a 20-μM concentration and at a level of depolarization near the threshold for triggering of action potentials.

Previous studies of mechanisms of burst onset in CA1 hippocampus (Fujita 1975; Wong and Prince 1981) have suggested a close relationship between sADPs and bursts. This notion is also supported by the data reported here. First, during the carbachol-induced depolarization and the increase of firing frequency in LSml neurons, the appearance of sADPs preceded the occurrence of the first spontaneous bursts. Second, a sADP could always be observed after action potentials evoked at the RMP by depolarizing current
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FIG. 10. Blockade of CCh-induced responses in low Na⁺ medium. A, left trace: response to CCh in a type I neuron. Middle traces: progressive blockade of a type I burst in low Na⁺, but action potentials remained. Right trace: recovery of the type I burst. B, top and middle left traces: response to CCh in a type II neuron. Top right trace: progressive blockade of a rhythmic bursting discharge in low Na⁺, but action potentials remained (middle right trace). Bottom left trace: recovery of a rhythmic bursting discharge of type II (gap duration: 60 s). Bottom right trace: expanded record of a type II burst during the recovery. C, left trace: response to CCh in a type I neuron. Middle trace: a sADP (X) was observed in the presence of CCh/TTX. Right trace: coinjection of low Na⁺ blocked the sADP (X).

pulses in neurons showing bursting discharges elicited by carbachol. Third, abating an evoked burst with a hyperpolarizing current always disclosed an underlying sADP. Fourth, these sADPs from successive spontaneous or evoked spikes progressively increased in amplitude to provide a depolarizing drive capable of inducing bursting activity. Fifth, spontaneous and evoked sADPs and bursts were modulated by changes in membrane potential, these two events being suppressed by hyperpolarization. Sixth, sADPs and bursts were dose dependent. Finally, spontaneous and evoked sADPs and bursts displayed an equivalent ionic dependence and pharmacological profile. Based on this relationship, it was hypothesized that sADPs associated with spontaneous or evoked action potentials play a central role in the onset of the bursting discharges induced by carbachol in LSml neurons.

The muscarinic induction of a sADP was previously observed in cortical neurons (Andrade 1991; Araneda and Andrade 1991; Constanti and Bagetta 1991; Constanti et al. 1993; Libri et al. 1994; McCormick and Prince 1986; Schwindt et al. 1988), in hippocampal neurons (Benardo and Prince 1982; Caeber et al. 1993), and in amygdaloid basolateral neurons (Washburn and Moises 1992). In CA1 pyramidal hippocampus (Benardo and Prince 1982), the sADP sometimes triggered a burst of action potentials. In cortical (Andrade 1991; Schwindt et al. 1988) and amygdaloid (Washburn and Moises 1992) neurons, the sADPs had the property of summation and evoked a prolonged repetitive firing. However, in these neurons, the induction of sADP and its summation were not accompanied by a rhythmic bursting discharge (Andrade 1991; Schwindt et al. 1988; Washburn and Moise 1992). In these neurons, the induction of a sADP accompanied the reduction of the slow AHP. In our study, the apamin-sensitive sAHP (Carette 1994) was not reduced by carbachol. A similar observation has been reported in cortical neurons (Schwindt et al. 1988) and locus coeruleus neurons (Osmanovic and Shefner 1993). In LSml neurons, the apamin-sensitive sAHP did not summate (Carette 1994), whereas sADPs induced by carbachol progressively increased. The conjunction of these two features easily allow spike threshold to be reached in type I neurons and plateau potential threshold in type II and III neurons.

Although there are several reports of sADPs after muscarinic stimulation as indicated above, one study has shown the cholinergic induction of plateau potentials in hippocampal CA1 pyramidal neurons (Fraser and MacVicar 1996). These plateau potentials showed common features with those described here and in our previous study (Carette 1997): a genesis from sADPs, a blockade after application of atropine, a persistence after application of TTX, a dependence on the holding potential, and a blockade in low Na⁺.

In our study, carbachol-induced bursting discharges and
FIG. 11. Effects of 0 Ca\textsuperscript{2+} medium on the CCh-induced responses. A, left traces: superimposed traces showing the partial blockade of a CCh-induced type III burst (arrow) in 0 Ca\textsuperscript{2+} after 15 min (arrowhead). Note the blockade of the sAHP after the burst in 0 Ca\textsuperscript{2+}. Middle traces: total blockade of this type III burst after 30 min. Right trace: recovery of the type III burst. B, top left trace: response to a depolarizing current pulse in control. Note the presence of a sAHP after the pulse (arrowhead). Top middle trace: rhythmic bursting discharge of type II induced by CCh in the same neuron. Top right trace: expanded record of the onset of this burst. A sAHP (arrowhead) was present after the 1st action potential. Bottom left trace: response to the same depolarizing current pulse in 0 Ca\textsuperscript{2+}. Note the higher number of action potentials and the absence of a sAHP after the pulse (arrowhead). Bottom middle trace: coapplication of 0 Ca\textsuperscript{2+} did not block the rhythmic bursting discharge induced by CCh. Note the inactivation of action potentials in these conditions during the bursts. Bottom right trace: expanded record of the onset of the burst. The 1st action potential did not show a sAHP (arrowhead). C, left traces: a CCh-induced sADP in response to a 4-ms current pulse was blocked by coapplication of 0 Ca\textsuperscript{2+}. Right traces: in the same neuron, the CCh-induced plateau potential in response to a 100-ms current pulse was also blocked by coapplication of 0 Ca\textsuperscript{2+}. D, left traces: a CCh-induced sADP in response to a 4-ms current pulse was blocked by coapplication of 0 Ca\textsuperscript{2+}. Right traces: in the same neuron, the CCh-induced plateau potential in response to a 400-ms current pulse was not blocked by coapplication of 0 Ca\textsuperscript{2+}.

sADPs were blocked by 1) substitution of extracellular Na\textsuperscript{+} by Tris in all the tested neurons and 2) Ca\textsuperscript{2+}-free medium or bath applying the Ca\textsuperscript{2+} channel blockers Co\textsuperscript{2+} or Ni\textsuperscript{2+} in about one-half of neurons. Taken together, these results suggest that in LSml neurons, carbachol induces sADPs and bursts by a cationic conductance largely permeable to Na\textsuperscript{+} ions and that in some LSml neurons, a Ca\textsuperscript{2+} influx is required for activating or controlling muscarinic receptor stimulation.
FIG. 12. Effects of tetraethylammonium (TEA) and Cs⁺ on CCh-induced rhythmic bursting discharges and sADPs. A: traces were chart records of the rhythmic bursting discharge induced by CCh in a type III neuron. Coapplication of 1 mM TEA blocked the rhythmic bursting discharge that recovered after 6 min. Bottom right trace: expanded oscilloscope record of spontaneous activity in the presence of CCh and TEA. Note the broadening of the 2nd action potential. B, top left trace: response to a 100-ms current pulse in control. Top middle trace: after application of 1 mM TEA, the 2nd action potential was prolonged and a sADP appeared. Top right traces: superimposed records of a plateau potential induced by CCh and a sADP observed in the presence of CCh and TEA. A plateau potential was not evoked by CCh in the presence of TEA (compare with middle trace for the sADP). Bottom left trace: in the same neuron, no postpotential was observed after 1 evoked action potential in control. Bottom middle trace: after application of 1 mM TEA, a small sADP appeared. Bottom right traces: superimposed records of sADPs. The CCh-induced sADP was blocked by TEA. Calibrations in B also apply in C except for time (C, right traces). C, left trace: response to a hyperpolarizing current pulse in control. Note the depolarizing sag (indicated by an arrow) produced by the pulse. Middle trace: 2 mM Cs⁺ completely blocked the sag. Right traces: in the same neuron, superimposed records of rhythmic bursting discharges induced by CCh (type III neuron). In the presence of Cs⁺, the rhythmic bursting discharge persisted but the duration of the interburst interval was increased.

Effects. Recently, Caeser et al. (1993) and Fraser and McVicar (1996) have provided direct evidence that in hippocampal CA1 and CA3, muscarinic agonists activate a Ca²⁺-dependent cation current that is mainly carried by Na⁺ and that gives rise to an ADP and long-lasting plateau potentials. On the other hand, in locus coeruleus (Shen and North 1992) and in hippocampus (Guérineau 1995), muscarine activates a cationic current that is not Ca²⁺ dependent. Thus our observation that some LSml neurons still exhibited plateau potentials or rhythmic bursting discharges in zero Ca²⁺ suggests a role for Na⁺ in the initiation of bursts. In our study, TEA was found to inhibit the muscarinic-induced rhythmic bursting discharges and sADPs. The present observation is consistent with other studies in neuronal (Shen and North 1992) and nonneuronal cells (Inoue and Kuriyama 1991), indicating that TEA can block a cationic current induced by muscarine. This could be explained by the ability of TEA to block muscarinic receptors. However, it has been reported that low TEA concentrations (1–2 mM), also used in our study, do not block muscarinic receptors in neuroblastoma × glioma.
hybrid cells (Caulfield 1991). Thus it has been suggested that TEA may act by occluding the muscarinic receptor-activated cation channel (Chen et al. 1993). TEA may be attracted to a negatively charged binding site in the channel and thus interfere with the permeation of cations (Hille 1992). Our results show that Cs⁺ did not block sADPs and bursts induced by carbachol. Moreover, our results suggest that Iₑ₉ present in LSm1 (Carette et al. 1992) may participate in rhythmic bursting discharges by counterbalancing prolonged hyperpolarizations observed with some discharges.

The physiological significance of the cholinergic containing pathway originating from the tegmentum is still speculative. Injections of acetylcholine (ACh) into the lateral septum caused blood pressure increases in rats (Peres-Polon and Correa 1994). In addition, the response to ACh was blocked by hypophysectomy, further suggesting a major involvement of circulating vasopressin (Peres-Polon and Correa 1994). The vasopressinergic neurons in the supraoptic nucleus and paraventricular nucleus receive a large input of fibers originating in the LS (Bailey and Nurnberger 1991; Staiger and Wouterlood 1990). Excitatory and inhibitory inputs from the LS to vasopressinergic neurons have been reported using electrophysiological methods (Poulain 1983). Vasopressinergic neurons possess endogenous mechanisms underlying phasic activity modulated by synaptic activity (Legendre and Poulain 1992). Phasic activity in these neurons occurs in response to cardiovascular stimuli and has a direct impact on the pattern of vasopressin release (Cazalis et al. 1985). It is thus tempting to speculate that in our study, LSm1 neurons responsive to carbachol by a rhythmic bursting discharge control directly or indirectly the phasic activity of vasopressinergic neurons to induce hormone release in response to appropriate physiological cardiovascular stimuli.

Tetanic stimulation of the fimbria has been found to induce a long-term potentiation (LTP) in the mediolateral part of the LS (Garcia et al. 1996). This synaptic enhancement depended on the activation of the NMDA subtype of glutamate receptors (Stevens and Cotman 1991; Van Den Hooff et al. 1989). In the CA1 area of the hippocampus, it has been shown that a postsynaptic depolarization is required to remove the magnesium block of N-methyl-D-aspartate (NMDA) receptor and to induce the NMDA receptor-dependent LTP (Malenka and Nicoll 1993). A facilitation of the LTP induction by cholinergic systems has been suggested in Schaffer collateral-CA1 pathways (Blitzer et al. 1990) and in the fimbria-CA3 pathway of the hippocampus (Katsuki et al. 1992). In our study, muscarinic receptor activation might contribute to LTP induction in the LSm1, providing the postsynaptic depolarization required to remove the voltage-dependent magnesium block of NMDA receptors. Moreover, bursts appear to have a special role in synaptic plasticity. Thus, in the hippocampus, bursts can produce long-term synaptic modifications (Lisman 1997). In our study, rhythmic bursting discharges induced by carbachol in LSm1 neurons might also contribute to LTP induction in this area.

Our results support the idea that cholinergic afferents to the LSm1 establish direct contacts on the three classes of these neurons. The different rhythmic bursting discharges generated by carbachol (types I, II, and III) displayed the same characteristics in each class of neuron. Thus the sADP was the one contributor to the bursts in type I neurons. A depolarizing drive, i.e., plateau potential, depending on the sADPs and their increase in amplitude was the contributor to the bursts in type II and III neurons. In conclusion, in the three classes of neurons, carbachol produced qualitatively equivalent responses, and the mechanisms of action of the cholinergic agonist are identical for the three.

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