Angiotensin II Induces Calcium-Dependent Rhythmic Activity in a Subpopulation of Rat Hypothalamic Median Preoptic Nucleus Neurons

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Spanswick, David and Leo P. Renaud. Angiotensin II induces calcium-dependent rhythmic activity in a subpopulation of rat hypothalamic median preoptic nucleus neurons. J Neurophysiol 93: 1970–1976, 2005; doi:10.1152/jn.00769.2004. Whole cell patch-clamp recordings revealed a subpopulation (16%, n = 18/112) of rat median preoptic nucleus (MnPO) neurons responded to bath-applied angiotensin II (Ang II; 100 nM to 5 μM; 30–90 s) with a prolonged TTX-resistant membrane depolarization and rhythmic bursting activity. At rest, cells characteristically displayed relatively low input resistance and negative resting potentials. Ang-II-induced responses featured increased input resistance, a reversal potential of −95 ± 2 mV, an increase in action potential duration from 2.9 ± 0.5 to 4.3 ± 0.8 ms, and the appearance of a rebound excitation at the offset of membrane responses to hyperpolarizing current injection. The latter was sensitive to Ni2+ (0.5–1 mM; n = 5), insensitive to extracellular Cs+ (1 mM, n = 5), and intracellular QX-314 (4 mM, n = 5), consistent with activation of a T-type Ca2+ conductance. Coincident with the Ang-II-induced depolarization was the appearance of rhythmic depolarizing shifts at a frequency of 0.14 ± 0.09 Hz with superimposed bursts of 4–22 action potentials interspersed with silent periods persisting for >1 h after washout. These TTX-resistant depolarizing shifts increased in amplitude and decreased in frequency with membrane hyperpolarization with activity ceasing beyond approximately −80 mV, and were abolished in low-Ca2+/high-Mg2+ bathing medium (n = 6), Co2+ (1 mM; n = 6), or Ni2+ (0.5–1 mM; n = 8). Thus in a subpopulation of MnPO neurons, Ang II induces “pacemaker-like” activity by reducing a K+–dependent leak conductance that contributes to resting membrane potential and promoting of Ca2+-dependent regenerative auto-excitation mediated, in part, by a T-type Ca2+ conductance.

INTRODUCTION

The lamina terminalis, the thin strip of neural tissue forming the rostral wall of the third cerebral ventricle, contains three collections of neurons that serve a critical role in maintaining body fluid and cardiovascular homeostasis (reviewed in Johnson et al. 1996; McKinley et al. 1999). Two of these are circumventricular organs or CVOs the fenestrated capillaries of which permit blood-borne molecules access to central neurons. Located dorsally is the subfornical organ (SFO) the neurons of which are a target for circulating angiotensin; at the ventral edge of the lamina terminalis is the organum vasculosum lamina terminalis (OVLT) the neurons of which participate in osmoreception. Thus neurons in both of these CVOs serve a sensory role to systemic challenges such as a rise in plasma angiotensin (Ang II) or an increase in plasma osmolality, respectively. The consequences of their participation to these challenges is the instigation of specific responses. In the case of Ang II, peripheral or central administration results in a clearly defined change in behavior (e.g., drinking) as well as autonomic (e.g., suppression of sweating to eliminate evaporative heat loss) and/or endocrine reflexes (e.g., vasopressin release) aimed at the conservation and restoration of fluid volume (Lind and Johnson 1982; Owens et al. 1989).

While the importance of these lamina terminalis CVOs in these responses to Ang II is well characterized, the neural mechanisms underlying these actions rely on a third neuronal structure, the median preoptic nucleus (MnPO, also called MnPN or nucleus medianus). Located around the anterior commissure at the midpoint of the lamina terminalis, the MnPO has features suggestive of an integrating center. Its neurons are reciprocally connected with SFO and OVLT neurons, receive input from medullary autonomic neurons, and project to hypothalamic and brain stem targets that are known to regulate fluid balance (e.g., supraoptic and paraventricular magnocellular neurons) and cardiovascular function (e.g., nucleus tractus solitarii, ventrolateral medulla) (reviewed in McKinley et al. 1999). Moreover, MnPO is essential for the drinking response induced by central and peripheral administration of Ang II, as lesions of this nucleus abolish these responses (Cunningham et al. 1991). A critical component of the response to peripheral Ang II is the SFO the neurons of which are not only sensitive to blood-borne Ang II but also give rise to an angiotensin-immunoreactive innervation to MnPO (Lind et al. 1984). The presence within MnPO of a high-density of angiotensin binding sites, as well as neurons expressing AT1 type receptors (Lenkei et al. 1998) and responding with excitation and membrane depolarization to exogenous angiotensin (Bai and Renaud 1998; Travis and Johnson 1993), suggest that this nucleus has a pivotal role in coordinating the central drive to drink induced by elevated levels of circulating Ang II. However, to date there is little information on the cellular mechanisms whereby this peptide modulates activity of MnPO neurons to bring about such behavioral changes.

To address this issue, we have utilized whole cell patch-clamp recording techniques in an in vitro hypothalamic slice preparation to investigate the actions of Ang II on MnPO neurons. An earlier study (Bai and Renaud 1998) revealed that ~50% of neurons responded to exogenous Ang II with an AT1 receptor-mediated prolonged depolarization and associated re-
duction in membrane conductance. We now report that a subpopulation of MnPO neurons responding to a brief exposure to Ang II displays a unique pacemaker-like activity that persists for hours postinduction, a feature that is intrinsic to these neurons and conditional on exposure to Ang II. We therefore propose these neurons to be Ang II-sensitive “conditional pacemakers” the participation of which may be a key element in a central mechanism underlying the behavioral drinking response.

METHODS

Slice preparation

The methods employed in this study were in accordance with the principles and guidelines of the Canadian Council for Animal Care. Briefly, male Long-Evans rats (200–350 g body wt, 6–12 wk old) were anesthetized with halothane and decapitated and the brain was removed and stripped of dura and pia mater. Coronal or sagittal slices (300–450 µm) of hypothalamus containing the MnPO were cut on a vibratome under ice-cold, gassed (95% O2-5% CO2) artificial cerebrospinal fluid (ACSF) of the following composition (in mM) 127 NaCl, 1.9 KCl, 1.2 KH2PO4, 2.4 CaCl2, 1.3 MgCl2, 26 NaHCO3, and 10 D-glucose; pH 7.4. Slices were maintained carbogenated at room temperature for 1–2 h prior to transfer to a submerged-type recording chamber.

Recording

Whole cell recordings were obtained from MnPO neurons at room temperature and at 34 ± 0.5°C. Patch-pipettes with resistances of 3–12 MΩ were filled with an intracellular solution of the following composition (in mM) 130 Kgluconate, 10 KCl, 0.2 MgCl2, 0.1 CaCl2, 0.5 EGTA-Na, 10 HEPES, and 2 Na2ATP, pH 7.4 (with NaOH). Recordings were obtained with a patch-clamp amplifier (Axopatch 1D, Axon Instruments). Correction of the liquid junction potential was applied to whole cell recordings. Access resistances ranged between 5 and 30 MΩ. Recordings were monitored on an oscilloscope (Gould 1602), displayed on a pressure ink pen recorder (Gould RS3200), and stored on videotape for later off-line analysis. Statistical analysis was performed using Excel (Microsoft) with all values given as means ± SD. Statistical significance was determined using Student’s two-tailed paired t-test. P < 0.05 was taken to indicate statistical significance.

Pharmacology

Drugs made up as concentrated stocks, diluted in ACSF and applied by superfusion at known concentrations in the bathing medium, included: Ang II, the voltage-dependent sodium channel blocker tetrodotoxin (TTX; Sigma and/or Sigma/RBI, St Louis, MO), the calcium channel blockers Co2+ and Ni2+, and the nonselective potassium channel blockers Cs+ and Ba2+. QX-314 (Sigma), an intracellular sodium channel blocker (also known to block hyperpolarization-activated nonsellective cation channels) was included in the pipette solution. In experiments where low-Ca2+ and high-Mg2+ bathing medium were used, CaCl2 was omitted from the bathing medium, and the concentration of MgCl2 increased to 5.2 mM.

Cell identification and morphology

For unambiguous identification of MnPO neurons, the morphology and anatomical localization was examined by including the intracellular marker Lucifer yellow (dipotassium salt, 1 mg/ml, Sigma) in the patch-pipette solution. Methods for visualizing neurons after whole cell recording with Lucifer yellow have been described in detail previously (Pickering et al. 1991). Briefly, after recording, slices were fixed in 4% parafomaldehyde overnight, cleared in DMSO, and mounted between two coverslips for subsequent epifluorescent microscopy (Zeiss Axioscope).

RESULTS

Properties of MnPO neurons

Whole cell recordings from a total of 112 MnPO neurons situated ventral or dorsal to the anterior commissure revealed a collective mean resting membrane potential and input resistance of −56.6 ± 8.0 mV and 1,581 ± 668 MΩ, respectively. We observed no obvious differences in the morphology, electrophysiological properties, or sensitivity to Ang II of MnPO neurons in coronal versus sagittal slices.

Ang II induction of a pacemaker-like activity

During this study we noted that a subpopulation (16%, n = 18/112) of MnPO neurons were characteristically silent and responded to Ang II in a unique manner. These cells had a comparatively hyperpolarized mean resting membrane potential (−68.5 ± 8.0 mV; range: −78 to −58 mV) and low input resistance (1,068 ± 386 MΩ). Typically these cells responded to bath application of Ang II (100 nM to 5 µM, 10–90 s) with a slowly developing membrane depolarization (15.2 ± 6.3 mV) and rhythmic bursts of action potential discharge (Figs. 1, A and B). Rhythmic activity induced by Ang II was characterized by bursts of 4–22 action potentials interspersed with silent periods of inactivity. The mean frequency of these bursts was 0.14 ± 0.09 Hz at the final steady-state membrane potential induced by Ang II (data from 5 cells). Individual bursts displayed characteristic wave-forms comprising slow, progressive depolarizing shifts, with superimposed trains of action potentials toward the peak of the shift, followed by a relatively rapid termination and silent period before the onset of the subsequent burst (Fig. 1B). The mean peak amplitude of these depolarizing shifts was 19.1 ± 1.4 mV (calculation from 5 neurons at a holding potential of −70 mV). Burst firing induced by Ang II persisted even at membrane potentials more negative than the response resting membrane potential. As illustrated in Fig. 1B, bursts of action potential firing decreased in frequency with increasing membrane hyperpolarization and ceased at membrane potentials more negative than around −80 mV. These features suggest that this Ang-II-induced burst firing involves mechanisms that are intrinsic to these neurons. Indeed rhythmic activity persisted in the presence of TTX (0.5–1 µM; see Fig. 4, A2 and B2; n = 8).

Ang-II-induced responses were associated with a concomitant 34.9 ± 19.8% increase in neuronal input resistance to 1,377 ± 275 MΩ (P < 0.001, see Figs. 1A and 2A), assessed from measurements taken after manual repolarization of the membrane to prereceptor resting levels by injection of constant negative current (Fig. 2A). Voltage-current relations of MnPO neurons before and during exposure to Ang II indicated a reversal potential of −95 ± 2 mV (see Fig. 2, B and C; n = 5), close to the reversal potential for K+ ions under our recording conditions (E[kg−98 mV]. Ang-II-induced rhythmic activity was sustained over the time course of the experiment, ±4.5 h in some cases (n = 7), and partly recoverable in the remaining neurons. Quantitatively and qualitatively similar data were obtained in experiments performed at 34 ± 0.5°C in...
partly reduced by Ni$^{2+}$ at a concentration of 500 μM ($n = 3$) and completely blocked at a concentration of 1 mM ($n = 5$; Fig. 3A4). Extracellular Cs$^+$ (1 mM; $n = 7$; Fig. 3A3) and intracellular QX-314 (4 mM; $n = 5$; Fig. 3B) were without effect on the Ang-II-induced membrane rebound depolarization.

Ang II also significantly modulated the action potential waveform. When evoked in response to suprathreshold depolarizing current pulses (0.05–20 pA), action potential repolarization in the presence of Ang II (100 nM to 5 μM), was slowed and the duration increased from 2.9 ± 0.5 ms in control to 4.3 ± 0.8 ms with a significant “shoulder” appearing on the repolarizing aspect (Fig. 3C).

**Ionic mechanism underlying Ang-II-induced rhythmic activity**

To determine the mechanisms underlying the generation of Ang-II-induced rhythmic activity, we tested the effects of a range of ion channel blockers. Whereas the voltage-dependent sodium channel blocker TTX (1 μM, $n = 3$) blocked fast action potential discharge, rhythmic activity persisted in the form of slow-rising depolarizing shifts that gave rise to a single action potential (calcium spike) at the peak of the shift (Fig. 4A2). The frequency of the depolarizing shifts and associated spike was little affected by TTX, being similar to that of bursts seen prior to TTX (1.2 ± 0.3 Hz in TTX vs. 1.2 ± 0.4 Hz before TTX). Similar activity was induced in response to Ang II in each of three neurons preexposed to TTX.

To investigate the role of calcium in Ang-II-induced rhythmic activity, we tested the effects of reducing extracellular Ca$^{2+}$ concentration, the nonselective Ca$^{2+}$ channel blocker Co$^{2+}$, and the relatively selective T-type Ca$^{2+}$ channel blocker Ni$^{2+}$. Superoxen of nominally 0 mM Ca$^{2+}$ and high Mg$^{2+}$ (5.2 mM) bathing medium in TTX ($n = 6$) had several effects. At the onset of exposure to these conditions the frequency of spontaneous rhythmic activity increased, action potential amplitude decreased and the interburst intervals were progressively reduced. At the peak of the response, irregular shifts in membrane potential persisted, and the membrane potential remained 10–15 mV more depolarized than the peak of the membrane hyperpolarization observed during rhythmic activity. Rhythmic activity was therefore disrupted under these conditions (Fig. 4A, 2–4). On returning Ca$^{2+}$ and Mg$^{2+}$ to normal, activity was progressively restored, the frequency of activity decreasing with progressive restoration of normal Ca$^{2+}$ and Mg$^{2+}$ levels. Again, the frequency and timing of the rhythmic activity were re-established within 15 min of returning calcium to the bathing medium (Fig. 4A, 4 and 5).

Bath application of Co$^{2+}$ (1 mM) completely and reversibly abolished Ang-II-induced rhythmic activity (Fig. 4A6; $n = 6$). In the presence of TTX, the T-type Ca$^{2+}$ channel blocker Ni$^{2+}$ partly reduced rhythmic activity and underlying depolarizing shifts at a concentration of 0.5 mM ($n = 4$; Fig. 4B1-3) and completely blocked regenerative rhythmic activity induced by Ang II at a concentration of 1 mM (Fig. 4B4; $n = 8$), a concentration that blocked the T-type calcium conductance in these neurons (see Fig. 3A). The effects of these divalent cations were completely reversible, the frequency and timing of the rhythmic activity being re-established within 15 min of wash (Fig. 4, A7 and B5).
DISCUSSION

This study reports that a subpopulation of MnPO neurons characterized by a relatively hyperpolarized resting membrane potential and low input resistance responds to exogenous Ang II with membrane depolarization and a prolonged, bursting, “pacemaker-like” activity. The latter appears to be unique to these cells and involves a calcium-dependent regenerative activity and conductances that are intrinsic to the neurons, endowing them with a capability for autorhythmic activity, conditional on exposure to Ang II.

Mechanism of Ang-II-induced excitation

Ang-II-induced membrane depolarization and burst firing activity was associated with a decrease in neuronal input resistance. A: current-clamp trace illustrates Ang-II-induced membrane depolarization associated with an increase in neuronal input resistance, the latter indicated by the increase in the amplitude of electrotonic potentials (downward deflections of the record) evoked in response to hyperpolarizing current pulse injection (0.03 Hz, 500 ms, –20 pA). At the peak of the response, manual membrane hyperpolarization to prerelaxation resting levels revealed the increase in neuronal input resistance to be due to exposure to Ang II rather than an indirect effect of activation of voltage-dependent conductances.

FIG. 2. Ang-II-induced changes in passive membrane properties. A: current-clamp trace illustrates Ang-II-induced membrane depolarization associated with an increase in neuronal input resistance, the latter indicated by the increase in the amplitude of electrotonic potentials (downward deflections of the record) evoked in response to hyperpolarizing current pulse injection (0.03 Hz, 500 ms, –20 pA). At the peak of the response, manual membrane hyperpolarization to prerelaxation resting levels revealed the increase in neuronal input resistance to be due to exposure to Ang II rather than an indirect effect of activation of voltage-dependent conductances. Bottom: electrotonic potentials sampled from the points in the record above indicated by the arrows and shown here on a faster time base. Note the increase in peak amplitude of the potential elicited after exposure to Ang II compared with control, indicating an increase in neuronal input resistance. B: samples of a continuous record showing superimposed membrane responses to injection of current pulses in the absence (control) and after exposure to Ang II. C: plot of data shown in B. Note the increased slope in the presence of Ang II and point of intersection of the plots indicating the reversal potential of the Ang-II-induced depolarization around –95 mV. This is close to the reversal potential for potassium ions under our recording conditions.

FIG. 3. Effects of Ang II on intrinsic active conductances contributing to autorhythmicity. A: samples of a continuous current-clamp record showing superimposed electrotonic potentials evoked in response to hyperpolarizing rectangular-wave current pulses (0.10–0.35 pA; 500 ms; 0.03 Hz). A1: electrotonic potentials evoked prior to exposure to Ang II. A2: electrotonic potentials evoked in the presence of Ang II. Note the rebound depolarizations evoked at the offset of the response to current injection. A3: subsequent exposure to Cs+ was without significant effect. A4: however, Ni2+ (1 mM) completely blocked the rebound excitation. B: superimposed electrotonic potentials evoked in response to hyperpolarizing rectangular-wave current pulses (0.4 pA; 500 ms; 0.03 Hz) in a QX-314-loaded neuron prior to and during exposure to Ang II. Note the rebound excitation evoked at the offset of the membrane response to hyperpolarization in the presence of Ang II. Each record in this figure is the average of 8 consecutive electrotonic potentials. C: samples of a continuous record showing Ang-II-induced modulation of the action potential waveform. The action potential was prolonged in the presence of Ang II (2) compared with control (1) and a distinct shoulder on the repolarization phase exposed in the presence of Ang II. Each record here is the average of 8 consecutive action potentials evoked in response to depolarizing current injection.
conductance that could persist for >1 h after presumed washout of the agent. Because of the prolonged nature of the Ang-II-induced response, maintaining stable recording conditions until the establishment of a full recovery in combination with a tendency for desensitization, precluded a detailed pharmacological investigation of the concentration dependence and receptor specificity of the response. However, some of these aspects were featured earlier (see Bai and Renaud 1998). In the present investigation, current-voltage relations indicated a reversal potential of \(-95 \pm 2\) mV, close to the reversal potential for K\(^{+}\) ions under our recording conditions. These data imply that Ang-II-induced rhythmic activity, in part, involves block of one or more K\(^{+}\) leak conductances that contribute to resting membrane potentials in these neurons. Ang-II-induced reductions in potassium conductances have also been noted in other neurons (Li and Ferguson 1996; Li and Guyenet 1996; Nagamoto et al. 1995).

Intrinsic active conductances contributing to Ang-II-induced rhythmic activity

In addition to its effects on resting membrane conductances, Ang II also either revealed or modulated active conductances expressed by these neurons. In the presence of Ang II, a

FIG. 4. Ang-II-induced burst firing is mediated by an underlying Ca\(^{2+}\)-dependent regenerative mechanism. Samples of continuous records from 2 MnPO neurons are shown. A1: spontaneous burst firing induced by Ang II. A2: subsequent application of TTX abolished bursts of action potentials giving rise to regular, single action potential discharge. A3: in low-Ca\(^{2+}\)/high-Mg\(^{2+}\) bathing medium, the frequency of firing initially increased and ultimately activity was abolished, effects fully reversible on returning to normal calcium and magnesium levels (A, 4 and 5). A6: addition of a nonselective calcium channel blocker Co\(^{2+}\) completely abolished all activity, again effects reversible on washout of the cation (A7). B1: recordings from another neuron showing burst firing induced by Ang II. B2: in the presence of TTX, large amplitude "plateau-like" potentials persisted. This activity was partly reduced by addition of Ni\(^{2+}\) at a concentration of 0.5 mM (B3) and completely abolished in 1 mM Ni\(^{2+}\) (B4), effects reversible on washout of this cation (B5).
Ang-II-induced burst firing in MnPO

rebound excitation was observed at the offset of the response to membrane hyperpolarization, giving rise to bursts of action potentials that were similar to those observed spontaneously after exposure to Ang II. This conductance was blocked by a low-voltage-activated (LVA) T-type Ca\textsuperscript{2+} channel blocker Ni\textsuperscript{2+}, and unaffected by Cs\textsuperscript{+} and intracellular QX-314, the latter two being blockers of the time-dependent hyperpolarization-activated inward rectifier \(I_h\) (Pape 1996; Robinson and Siegelbaum 2003). Thus in the presence of Ang II a T-type Ca\textsuperscript{2+} conductance was revealed or unmasked in MnPO neurons. Although the concentrations of Ni\textsuperscript{2+} required to block the rebound excitation were relatively high (>500 \(\mu M\)) other studies show variation in the sensitivity of T-type Ca\textsuperscript{2+} conductances to Ni\textsuperscript{2+}, (Lee et al. 1999; Wilson et al. 2002). This partly reflects the subunit composition of the channels. At present, the T-type Ca\textsuperscript{2+} channel family consists of three members, Ca\textsubscript{V}3.1, Ca\textsubscript{V}3.2, and Ca\textsubscript{V}3.3 (see Perez-Reyes 1999). Differential expression of these subunits and associated subunits can yield channels and conductances with distinct properties proposed to reflect specific neuronal functions. T-type channels are activated at potentials negative to threshold for Na\textsuperscript{+}-dependent action potential firing and activation of high-threshold Ca\textsuperscript{2+} conductances and act to amplify sub-threshold stimuli leading to suprathreshold activity by providing a depolarizing drive (see Huguenard 1996). These features are thought to be fundamental to the initiation of firing and in generating pacemaker-like activity in certain neuronal types and in synchronization of network activity (Huguenard and McCormick 1992; Llinas 1988). Ang II also slowed the repolarization phase of the action potential, extending its duration and forming a distinct “shoulder” on the repolarizing aspect. This is consistent with a component of the spike being mediated via high-threshold calcium conductances. Ca\textsuperscript{2+} components of the action potential in MnPO neurons were subsequently revealed by their sensitivity to compromised trans-membrane Ca\textsuperscript{2+} flux in the presence of TTX. The most-likely explanation for the broadening of the action potential and unmasking of the shoulder is block of K\textsuperscript{+} conductances. Similar modulatory roles for neurotransmitters have been reported in other neurons, e.g., sympathetic neurons (Spanswick et al. 1995; Yoshimura et al. 1986). Functionally, in these MnPO neurons, the broadening of the action potential may enhance Ca\textsuperscript{2+} influx and thus contribute to the calcium-dependent regenerative mechanism underpinning the pacemaker-like activity. Further experiments are required to clarify this issue.

Ang-II-induced calcium-dependent regenerative pacemaker activity

Ang-II-induced rhythmic activity persisted in the presence of TTX, suggesting a direct effect on the cell. Furthermore, Ang-II-induced burst firing was sensitive to changes in membrane potential, being progressively reduced with membrane hyperpolarization to around ~80 mV, beyond which activity ceased. Taken together, these data suggest burst firing induced by Ang II in MnPO neurons is generated by mechanisms intrinsic to the cell rather than through activation of local extrinsic synaptic circuits.

A role for calcium was clearly demonstrated by the fact that Ang-II-induced rhythmic activity was selectively blocked or abolished by maneuvers to inhibit calcium entry into the cell either by exposure to low-calcium/high-magnesium bathing medium or to the nonselective Ca\textsuperscript{2+} channel blocker Co\textsuperscript{2+}. These data are consistent with Ang II inducing or unmasking a Ca\textsuperscript{2+}-dependent regenerative mechanism that underlies the generation of rhythmic burst firing in these neurons. Furthermore, the appearance of low-threshold T-type conductances in the presence of Ang II supports the notion that the peptide can induce or unmask a regenerative calcium-dependent mechanism that in part involves activation of a T-type Ca\textsuperscript{2+} conductance. This in turn provides the driving force for activation of Na\textsuperscript{+} and high-threshold Ca\textsuperscript{2+} conductance activation. These data therefore suggest that a subpopulation of MnPO neurons express ionic conductances that predispose them to electrical autorhythmicity, conditional on exposure to Ang II. The role of Ang II appears to be as “a trigger” because once activated rhythmic activity may persist for several tens of minutes to hours after washout.

While it remains to be clarified as to how Ang II brings about this shift to autorhythmic electrical activity, blockade of a resting membrane leak conductance appears to be pivotal. We suggest that resting membrane K\textsuperscript{+} conductances act as a “shunt” to suppress intrinsic oscillatory conductances. Ang II effectively removes this shunt and modulates spike repolarization leading to increased Ca\textsuperscript{2+} influx and Ca\textsuperscript{2+}-dependent autorhythmicity. However, we cannot discount the possibility that other mechanisms, including direct modulation of calcium conductances, may be involved.

Ang-II-induced burst firing described here is the product of intrinsic membrane conductances that create a slow depolarizing wave that triggers multiple spikes. Mechanisms for generating slow depolarizing shifts vary among cell types and can involve calcium spikes (White et al. 1989), nonactivating sodium conductances (Azouz et al. 1996; Jensen et al. 1996), low-threshold calcium conductances (Llinas and Yarom 1981; van Den Top et al. 2004), and the hyperpolarization-activated nonselective cation conductance \(I_h\) (Pape 1996; Robinson and Siegelbaum 2003). The ability to generate burst firing is also not a fixed feature of neurons as changes in resting membrane potential required for low-threshold calcium and \(I_h\) activation (Steriade and Llinas 1988), changes in extracellular potassium concentration (Jensen et al. 1994) and neurotransmitters (Azouz et al. 1994; Wang and McCormick 1993) affect the ability of a neuron to generate bursts. The situation described in these MnPO neurons is reminiscent of that previously described in the inferior olive (Jahnsen and Llinas 1984; Llinas and Yarom 1986) and arcuate nucleus of the hypothalamus (van Den Top et al. 2004).

Functional implications

Increases in plasma and cerebrospinal fluid osmolality, and circulating Ang II levels can instigate a series of responses aimed at the conservation and restoration of fluid volume. These include drinking, elevations in vasopressin release, and a suppression of sweating to eliminate evaporative heat loss. The outcome of earlier lesion studies reveal that the MnPO is essential for the drinking response induced by central and peripheral administration of Ang II and has a pivotal role in coordinating the central drive to drink induced by elevated levels of Ang II (Cunningham et al. 1991). Neurons with
autorhythmic electrical oscillatory properties are thought to be important in many functional roles and behaviors including sleep-wakefulness, attention, motor co-ordination, development of neural connections, and learning and memory (see Llinas 1988). Recent experiments suggest ‘bursts’ may be a more efficient and reliable means of neuronal communication of information (see Lisman 1997). Data presented here suggest that activation of neurons in MnPO by Ang II induces prolonged autorhythmic electrical activity that may underlie the central cellular mechanism resulting in behavioral changes appropriate to the restoration and conservation of body fluid homeostasis. The fact that these neurons represent a subpopulation of MnPO neurons suggests functional specialization. This may reflect a specific chemical phenotype of MnPO neuron or neurons with a target and function-specific organization. In relation to this point, recent studies in the arcuate nucleus revealed orexigen-sensitive NPY/AgRP pacemaker-like cells similar to those described here (van Den Top et al. 2004) that may play a pivotal role in the central drive to feed. It is interesting to speculate that conditional pacemakers such as these may form component parts of conserved central pattern generators underpinning the central drive to drink in the case of the MnPO and to feed in the case of the arcuate nucleus. Further studies are required to fully appreciate the functional significance of these conditional pacemaker neurons.

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GRANTS

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