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Activity-dependent feedback modulation of spike patterning of supraoptic nucleus neurons by endogenous adenosine

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Bull, P. M., C. H. Brown, J. A. Russell, and M. Ludwig. Activity-dependent feedback modulation of spike patterning of supraoptic nucleus neurons by endogenous adenosine. Am J Physiol Regul Integr Comp Physiol 291: R83–R90, 2006. First published February 23, 2006; doi:10.1152/ajpregu.00744.2005.—Neuropeptide secretion from the dendrites of hypothalamic magnocellular supraoptic nucleus (SON) neurons contributes to the regulation of neuronal activity patterning, which ultimately determines their peptide output from axon terminals in the posterior pituitary gland. SON dendrites also secrete a number of other neuromodulators, including ATP. ATP degrades to adenosine in the extracellular space to complement transported adenosine acting on pre- and postsynaptic SON A1 receptors to reduce neuronal excitability, measured in vitro. To assess adenosine control of electrical activity in vivo, we made extracellular single-unit recordings of the electrical activity of SON neurons in anesthetized male rats. Microdialysis application (retrodialysis) of the A1 receptor antagonist, 8-cyclopentyl-1,3-dimethylxanthine (CPT) increased phasic vasopressin cell intraburst firing rates progressively over the first 5 s by 4.5 ± 1.6 Hz (P < 0.05), and increased burst duration by 293 ± 64% (P < 0.05). Hazard function plots were generated from interval interspike histograms and revealed that these effects were associated with increased postspike excitability. In contrast, CPT had no effect on the firing rates and hazard function plot profiles of continuously active vasopressin and oxytocin cells. However, CPT significantly increased clustering of spikes, as quantified by the index of dispersion, in oxytocin cells and continuously active vasopressin cells (by 267 ± 113% and 462 ± 67%, respectively, P < 0.05). Indeed, in 4 of 5 continuously active vasopressin cells, CPT induced a pseudophasic activity pattern. Together, these results indicate that endogenous adenosine is involved in the local control of SON cell activity in vivo.

The spiking activity of many neurons in the central nervous system is composed of brief bursts of high-frequency discharge. In urethane-anesthetized rats, ~50% of hypothalamic supraoptic (SON) and paraventricular magnocellular neurosecretory vasopressin cells fire action potentials (spikes) in a “phasic” pattern composed of active and silent periods each lasting tens of seconds (4, 22), which increases secretion efficiency due to frequency facilitation of peptide release from the posterior pituitary gland. This spontaneous phasic activity in vivo is critically dependent upon randomly patterned postsynaptic potentials, predominantly from excitatory glutamate and inhibitory GABA afferent inputs (5, 13, 39). Phasic vasopressin cells have membrane properties that convert this random input into a rhythmic output. By contrast, nonphasic vasopressin and oxytocin cells fire in a continuous irregular pattern or remain silent under basal conditions.

The cell bodies of magnocellular neurons are mainly located in the paraventricular nucleus and SON, from which their axons project to the posterior pituitary gland to release their peptide products into the peripheral circulation to principally regulate reproduction and body fluid balance (22). Neuropeptide secretion also occurs from magnocellular cell dendrites (26, 33) and is reported to regulate the pattern and overall activity of vasopressin and oxytocin cells in the SON (4). For example, vasopressin and coreleased peptides secreted from vasopressin dendrites influence the organization of phasic activity (6, 7, 15, 25). Dynorphin, a κ-opioid peptide coreleased from vasopressin neurosecretory vesicles (7, 42), plays a major role in burst termination, while vasopressin regularizes local activity between neurons (6). Dendritic oxytocin secretion might also have a physiological function by increasing spike clustering, which underpins the bursting behavior of oxytocin cells in lactating rats (27, 28). Recent data indicate that some of the local actions of vasopressin and oxytocin are mediated by endocannabinoids (17).

Many other neuromodulators have been identified in SON neuron neurosecretory vesicles, including galanin (20), apelin (11), and ATP (31). Although ATP is excitatory to oxytocin and vasopressin neurons when applied to hypothalamic explants, these effects are truncated partly by rapid catabolism to adenosine in the extracellular space (18) and possibly by receptor desensitization (37). Adenosine is ubiquitous in the mammalian brain, acting through at least 4 G-protein-coupled receptors (A1, A2A, A2B, and A3) with both inhibitory and excitatory actions in several brain areas (9). Although there is evidence for the existence of all four receptor subtypes in the

Address for reprint requests and other correspondence: M. Ludwig, Centre for Integrative Physiology, Univ. of Edinburgh, George Square, Edinburgh EH8 9XD, UK (e-mail: mike.ludwig@ed.ac.uk). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
SON (29), the majority of adenosine effects reported to date are mediated via inhibitory A1 receptors (32, 34).

A1 receptor activation reduces calcium currents in dissociated SON neurons through the modulation of voltage-dependent calcium channels, which are crucial to neuronal activity (29). Other effects include inhibition of GABA and glutamate release via presynaptic receptors, a reduction in action potential duration, and powerful hyperpolarizing actions through postsynaptic receptors in vitro (30, 32). Therefore, we undertook a series of experiments to determine the functional contribution of endogenous adenosine to the control of magnocellular neuron activity patterning during extracellular electrophysiological single-unit recordings from the SON of urethane-anesthetized male rats.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Bantin and Kingman, Hull, UK) were kept under standard conditions (12:12-h light-dark cycle; ambient temperature 22 ± 1°C) with continuous access to food and water. All procedures were performed in accordance with the 1986 UK Animals (Scientific Procedures) Act and associated guidelines.

Electrophysiology. Rats were anesthetized with urethane (1.25 g/kg ip, Sigma), the left femoral vein and trachea were cannulated, and the pituitary stalk and right SON were exposed transpharyngeally (24). The 2-mm-long membrane of a locally made U-shaped microdialysis probe (25) was positioned onto the exposed ventral glial lamina of the SON. A glass micropipette filled with 0.15 M NaCl (20 – 40 MΩ) was introduced into the center of the dialysis probe loop to record extra- cellular single-unit activity within the SON (25) via a CED 1401 interface attached to a PC running Spike2 software (Cambridge Electronic Design, Cambridge, UK). A side-by-side stimulating electrode (Snej-200X, Clarke Electromedical Instruments, Reading, UK) was placed on the pituitary stalk and set to deliver single, matched biphasic pulses (1 ms, <1 mA peak to peak) for antidromic identification of SON neurons. Collision of antidromic action potentials, triggered by spontaneous orthodromic action potentials, and constant latency were subsequently used to confirm identification. Oxytocin neurons were distinguished from continuous vasopressin neurons by the transient excitation of the former in response to intravenous cholecystokinin [20 μg/kg iv; (35)]; phasically firing neurons were assumed to be vasopressinergic. Artificial cerebrospinal fluid (aCSF) was dialyzed at 3 μl/min throughout the experiment (pH 7.2, composition in mM: 138 NaCl, 3.36 KCl, 9.52 NaHCO3, 0.49 NaHPO4, 1.26 CaCl2, 1.18 MgCl2, and 2.16 urea).

A 10-mM stock solution of 8-cyclopentyl-1,3-dimethylxanthine (CPT) was dissolved in aCSF containing 0.1N NaOH and serially diluted in aCSF to working solutions containing 1, 0.1, and 0.01 mM CPT. Each concentration was dialyzed consecutively in increasing (CPT) was dissolved in aCSF containing 0.1N NaOH and serially dialyzed at 3 μl/min after a stable baseline period of recording had been obtained (after iv CCK administration, where given). On the basis of previous experiments, we estimate the concentrations of drugs achieved within the SON using this method to be 1 × 10−3 of that in the dialysate (25). We have previously reported that similar retrodialysis application of aCSF containing 1N NaOH has no consistent effect on the activity of phasic vasopressin neurons (5). The aCSF used here contained NaOH at 10−7–10−4 N and is expected to be similarly inert.

Phasic burst identification. Phasic bursts were identified using the “bursts” script in Spike2, with the following parameters as previously described (7): minimum 20 spikes/burst, minimum burst duration of 5 s, minimum interval between bursts of 5 s, maximum interval between spikes in any one burst of 1 s, and maximum initial interval to identify the start of a burst of 1 s. These parameters identified 342 bursts in the eight phasic cells recorded for these experiments (30 to 64 bursts per cell).

Hazard function. We investigated the effects of adenosine receptor antagonism on the organization of electrical activity in the SON by calculating hazard functions for each cell. Hazard functions quantify the changes in postspike excitability of neurons following spontaneous action potentials (23). Interspike interval histograms were constructed in 10-ms bins, using Spike2, for the basal firing rate periods and for the 30-min that each concentration of CPT was retrodialyzed onto the SON. The probability of spike firing (hazard) was calculated from the interspike interval histogram of individual cells using the formula: hazard = ni / (nt · ni-1), where ni is the number of events per interval, ni-1 is the total number of events preceding the current interval and nt is the total number of events in all intervals. This gives the inferred probability (as a decimal) of a cell firing a subsequent spike in any interval after a spike (at time 0) (23, 35). A constant hazard is proportional to the neuronal firing rate and is produced by a completely random firing pattern associated primarily with afferent driven activity. Divergence above or below a constant hazard level reveals periods of increased and decreased postspike excitability, respectively, which may result from changes in intrinsic membrane properties.

Peak early-to-mean late hazard ratio. The peak early-to-mean late hazard ratios were calculated from these hazard plots. To compare with previous analysis, we used the peak early hazard as the maximum hazard value reached within the first 70 ms after a spike (35). This is the period most likely to encompass the maximal effects of postspike hyperpolarizing and depolarizing membrane currents in all cell types investigated here (35). This was compared with the mean late hazard from 400 to 500 ms in phasic cells and 300 to 400 ms in continuous cells, which represented steady-state hazard in each cell type. This allowed us to quantify changes in hazard function associated with intrinsic membrane properties (early) while correcting for fluctuations in synaptic input (late).

Index of dispersion of firing rate. The index of dispersion of firing rate (idfr) is a measure of the variability of activity calculated from the standard deviation of the firing rate (sdfr) corrected for the mean firing rate (mfrr) in 4-s bins (sdfr/mfrr, Ref. 8). The idfr is similar to, but distinct from the coefficient of variation (100 sdfr/mfrr), in that a random firing pattern would be expected to have an idfr of 1, but with more clustered activity, idfr would be >1 and with more regular activity <1 (8). An increase in idfr has been reported to precede burst firing of oxytocin cells in lactating rats and is also correlated with the frequency and amplitude of bursting behavior (8). We analyzed a 10–20 min basal period and a 20-min period at the end of each CPT exposure from continuous cells. The idfr of phasic activity was calculated similarly but using only within-burst activity before and during 1 mM CPT administration. To allow for a steady-state adenosine effect to be reached, this analysis was conducted from 12 s onward in each burst.

Statistical analysis. A total of 18 cells were recorded and analyzed, each from a different rat. All group data are expressed as the means ± SE. To analyze the effects of 0.01, 0.1, and 1 mM CPT on individual cell types, we used one-way repeated-measures ANOVA (RM ANOVA), and where a significant difference was revealed, this was followed by pairwise post hoc analysis using Dunnett’s method. To compare the effects of CPT on two or more groups, two-way RM ANOVA was used followed by post hoc analysis using Student-Newman-Keuls method to isolate significant (P < 0.05) differences (SigmaStat software, SPSS Science, Chicago, IL). The change in firing rate between basal and 1 mM CPT treatment with time in phasic cells was analyzed with the Pearson product moment correlation. Results were considered significantly different at P < 0.05.
RESULTS

Adenosine modulates phasic activity. To determine the effects of endogenous adenosine on basal SON magnocellular neuron activity, rats were prepared for electrophysiology, as described above. Fig. 1A demonstrates the typical effects of sequentially increasing doses of CPT on the activity of a phasic vasopressin cell from a total of eight spontaneously active phasic cells similarly treated. The average basal firing rate of these cells was measured over a 10- to 20-min segment of stable activity and compared with their activity over a 20-min period at the end of each CPT exposure. During application of CPT, the overall firing rate of phasic cells increased from a basal value of 3.83 ± 1.16 to 6.35 ± 1.31 and 7.59 ± 1.21 spikes/s upon exposure to 0.1 and 1 mM CPT, respectively (n = 8, P < 0.05 RM ANOVA followed by Dunnett’s post hoc analysis, data not shown).

Because CPT was administered in serially increasing doses over 90 min (30 min for each dose), we cannot exclude the possibility that the effects of CPT might be partially time dependent. However, as described above, 10- to 20-min segments of activity were analyzed at the end of each 30-min period of CPT administration, when changes in activity had apparently reached steady state in the presence of each dose of CPT, suggesting that each sequentially increasing dose of CPT induced a (additive) dose-dependent increase in the firing rates of the cells recorded.

The intraburst firing rate, burst duration, and interburst intervals were also calculated. This revealed that the overall increase in firing rate comprised both an increased intraburst firing rate (from 7.23 ± 0.87 spikes/s to 9.8 ± 1.16 spikes/s) and burst duration (from 51.7 ± 18.7s to 117.5 ± 31.1s) upon exposure to 1 mM CPT, (both P < 0.05 RM ANOVA , Fig. 1, B and C ). No significant change in the interburst interval was found (Fig. 1D).

To ascertain whether changes in postspike excitability could account for the observed effects of CPT, interspike interval (ISI) histograms were calculated using data generated by Spike2 (Fig. 2A). Plots of ISIs during basal activity in phasic cells followed the typical profile of these cells (35). One millimole CPT treatment increased the number of spikes generated by these cells. The shape of the ISI histogram is determined by changes in the postspike excitability of a neuron after each action potential; this relationship between interspike interval duration and postspike excitability is portrayed in plots of hazard functions, and these revealed that CPT induces an increase in postspike excitability in the period up to 0.3 s after the previous spike (Fig. 2B).

We quantified the increased postspike excitability by calculating the peak early-to-mean late hazard ratio. Phasic vasopressin cells (n = 8) had a basal early-to-late hazard ratio of 2.16 ± 0.19, which rose significantly to 2.98 ± 0.41 after exposure to 1 mM CPT. This resulted from a significant increase in the early hazard value from a basal value of 0.12 ± 0.01 to 0.17 ± 0.02 (P < 0.05, Fig. 2C) after 1 mM CPT with no change in late hazard from 0.05 ± 0.005 basal to 0.06 ± 0.005 after 1 mM CPT.

Activity-dependent effects of CPT. Analysis of intraburst firing rates in 1-s bins exposed activity-dependent effects of
The idfr was not affected by CPT treatment at $0.52 \pm 0.08$ to $0.55 \pm 0.08$ (control to 1 mM CPT).

CPT increases the irregularity of firing in continuously active vasopressin and oxytocin cells. Figs. 4A and 5A demonstrate the typical effects of increasing doses of CPT applied to continuously active vasopressin and oxytocin cells, respectively. By contrast to phasic cells, there were no significant effects of 0.01, 0.1, and 1 mM CPT on the overall firing rates of continuously active vasopressin cells ($n = 5$, Fig. 4B) or oxytocin cells ($n = 5$, Fig. 5B). However, there was a significant increase in the idfr of continuously active vasopressin cells from a basal value of $1.46 \pm 0.16$ to $6.75 \pm 1.42$ after 1 mM CPT treatment ($P < 0.05$, Fig. 4C), which involved the adoption of phasic-like activity (Fig. 4A). The Spike2 bursts analysis program was able to identify discrete bursts in the presence of CPT. However, these bursts of activity do not have the same characteristic profile as typical phasic vasopressin bursts, which have an early peak in firing rate followed by a decline to a slower steady state (see Fig. 1 and Ref. 35) and will be referred to as pseudophasic activity. Similarly, the idfr of oxytocin cells rose significantly from a basal level of $0.48 \pm 0.04$ to $1.28 \pm 0.65$ after 1-mM CPT dialysis ($P < 0.05$, Fig. 5C), but the clustering of activity was less marked, and no bursts were identified.

CPT did not change the profile of the hazard function, or the peak early-to-mean late hazard ratio, for either continuously active vasopressin cells (Fig. 4, D and E) or oxytocin cells (Fig. 5, D and E). Similarly, separate analysis from periods of CPT. Within the first 5–6 s of burst initiation, the firing rate of bursts recorded during CPT administration increased more rapidly than under basal conditions; thereafter, a constant difference was maintained throughout the burst (Fig. 3A; data from 10–30 s not shown).

The difference in average firing rate over the first 10 s of bursts under basal conditions and then in the presence of 1 mM CPT is plotted in Fig. 3B. Pearson product moment correlation shows a significant positive correlation between time and the CPT-induced difference in firing rate ($P = 0.038$, correlation coefficient 0.66) for the 10 s analyzed and highly significant correlation ($P < 0.00005$, correlation coefficient = 0.99) during the first 5 s of bursts. This indicates that adenosine generation is activity dependent, and its effects reach a steady state ~5–6 s after the start of each burst.

We also calculated the intraburst idfr, in 4-s bins, from 12 s onward under basal conditions and subsequently in 1 mM CPT.
pseudophasic activity in continuous vasopressin cells also revealed no change in the hazard function (data not shown). The peak early-to-late hazard ratios generated from the data in Figs. 4D and 5D produced basal ratios in continuous vasopressin (3.29 ± 0.8) and oxytocin (1.28 ± 0.2) cells, similar to those reported previously (35).

DISCUSSION

Here, we demonstrate that endogenous adenosine is an important autocrine modulator of SON spike patterning via activity-dependent activation of adenosine receptors in vivo. Our experiments revealed that activity-dependent adenosine generation is a major determinant of spike clustering in continuously active vasopressin and oxytocin cells and in the burst dynamics of phasic vasopressin cells.

After administration of the A1 receptor antagonist CPT, burst duration and intraburst firing rate of phasic vasopressin neurons increased significantly. Second-by-second analysis within bursts indicated that A1 receptor activation was absent at the onset of bursts but increased over the first 5 s of bursts and remained stable thereafter. This suggests that at basal activity levels, there is a progressive activation of A1 receptor mechanisms only during burst firing of phasic vasopressin cells. The simplest explanation for the effects of CPT is that endogenous extracellular adenosine accumulates in an activity-dependent manner during burst firing. However, because adenosine contributes to spike frequency adaptation (where firing rate peaks within the first few seconds of burst initiation and then declines to a steady state) and spike frequency adaptation increases as bursts progress, it is possible that endogenous adenosine is present at the onset of bursts, but its effects are not exposed until after activity-dependent activation of the AHP, which underpins spike frequency adaptation in vasopressin cells (19).

The putative effects of endogenous adenosine on the AHP might not be direct because exogenous adenosine reduces spike duration in vitro (32), which would reduce Ca2+ entry during each spike and could consequently diminish the activation of Ca2+-dependent conductances, such as those that underpin the AHP (and DAP), to reduce spike frequency adaptation.

Besides tuberomammillary fibers projecting to the SON, which are enriched in adenosine deaminase (a marker for adenosinergic neurons), specific adenosine projections to the SON have not been identified (36, 41). Thus we propose that the main source of extracellular adenosine is from the magnocellular neurons themselves via a combination of adenosine secretion and the rapid catabolism of exocytosed ATP (37). Activity-dependent release of adenosine and ATP from other sources or activation of companion inhibitory mechanisms cannot be excluded. For example, given the close proximity of
neuronal glia, reports of ATP and adenosine release from these cells and the proposed modulatory effects of neuronal-glia interactions all suggest an astrocytic component may also be involved (14, 21).

Feed-forward inhibition by ATP and/or ADP of the ecto-5'-nucleotidase, which converts AMP to adenosine, provides a rate-limiting step in this pathway (9). A functional consequence of ATP-mediated feed-forward inhibition has been described during purinergic rundown of neuron activity associated with swimming in Tritonia (10). Enhanced ATP release from recruited motor neurons during periods of high activity, such as an escape swimming episode, would thus produce an advantageous delay in adenosine-mediated activity inhibition. Hence, ATP-induced inhibition of adenosine production, during the high-frequency discharge at the start of each burst in vasopressin cells might explain the delay in enhancement of intraburst firing rate by CPT. A similar mechanism would also allow phasic magnocellular neurons to fire faster and with longer bursts in response to acute strong stimuli, such as that seen during hyperosmotic stimulation. In addition, extracellular accumulation of ATP and adenosine during periods of high activity may also lead to a synergistic purine receptor down-regulation (37).

The potential importance of adenosine as an autocrine modulator of phasic neuronal activity in magnocellular neurons can be ascertained by comparing results from previous experiments using the same methodology. The effects of V1 and k-opioid receptor antagonist retrodialysis in vivo is an ~1 Hz increase in intraburst firing rate, and burst duration is prolonged by ~120% (7, 25). Similarly retrodialysis of a nitric oxide synthase inhibitor, to block nitric oxide production by supraoptic neurons, produced a 1.78 ± 0.36 Hz increase in firing rate in vasopressin cells, although this may not reflect the intraburst effects in phasic cells (38). However, 1 mM CPT produced an overall increase in intraburst firing rate of ~2.6 Hz (maximum increase 4.45 ± 1.6 Hz at 5 s into a burst, Fig. 3B) and a 230% increase in burst length, suggesting that adenosine is a major contributor to activity-dependent restraint of magnocellular SON neurons under basal conditions. It remains to be determined how the influence of acute and chronic activity stimulants, such as hypertonicity or lactation, is modified by the actions of adenosine on magnocellular neurons in vivo and what effects the plasticity of adenosine restraint, as demonstrated in other neuronal systems, have on this.

Phasic vasopressin cells normally enter a period of quiescence after a spike due to a postspike hyperpolarizing afterpotential (HAP) (3) and a slower afterhyperpolarization (AHP) (2). This is followed by a period of hyperexcitability lasting 1–3 s, in vitro, as a result of a nonsynaptic depolarizing afterpotential (DAP) (1). Because spike frequency adaptation

Fig. 5. CPT does not alter firing rate of oxytocin cells but increases their variability of firing. A: example 10-min activity segments from the same oxytocin cell recording under control conditions then 0.01, 0.1, and 1 mM CPT administration in 1-s bins. B: there is no change in mean firing rate of oxytocin cells during CPT administration; however, there is a dose-dependent increase in the variability of firing of these cells (C), as shown by the increase in the index of dispersion (*P < 0.05). D: mean hazard plot profiles from control (○) and 1 mM CPT retrodialyzed (●) periods are not different. E: mean ± SE peak early-to-mean late hazard ratios under basal conditions and during CPT administration show no significant changes, suggesting there is no modification of postspike excitability by endogenous adenosine in this cell type.
is known to involve activation of the AHP (19) and postspike potentials are postulated to modulate postspike excitability (35), we constructed hazard function plots to highlight modifications in postspike excitability before and during CPT treatment. The hazard function characteristics reported here are all similar to previous reports from our laboratory (6, 35), including the notable differences between magnocellular oxytocin and vasopressin cells. Continuously active vasopressin cells have an earlier mode in the hazard/interspike interval histogram plots, which potentially reflect a more transient DAP. Hazard plots from oxytocin cells also show evidence of postspike AHP/HAPs, but no evidence of subsequent hyperexcitability associated with the DAP, although a small DAP can be exposed in oxytocin cells if the AHP is blocked pharmacologically in vitro (16). Therefore, if the effects of adenosine on postspike excitability involve a modulation of DAP dynamics and if the DAP is more prominent in phasic cells, this would explain why only the hazard function plots of phasic cells are significantly affected by CPT. Consistent with possible effects on the DAP, adenosine does not alter AHP amplitude in vitro (32). However, as postspike DAPs and AHP/HAPs overlap temporally, we cannot categorically confirm this conclusion with hazard function analysis in vivo.

It has recently been shown that exogenous adenosine reduces spike width and hyperpolarizes SON neurons in vitro (32). Hence, CPT might broaden spikes in our in vivo experiments to increase the activation of voltage- and calcium-activated currents, and it might depolarize the resting membrane potential to increase firing rate. Each of these effects could influence activity patterning in SON neurons. Spike broadening would be expected to increase AHP amplitude, which evidently does not occur because spike frequency adaptation (induced by AHP activation) is reduced by CPT application; potential effects of spike broadening on the AHP might be occluded by direct A1 receptor inhibition of calcium currents (29). A sustained depolarization would be expected to increase activity from burst onset (because membrane potential would be closer to spike threshold); again, this does not appear to occur because the effects of CPT on firing rate increase as bursts progress. Although we cannot exclude the possible contribution of such actions (on spike width and resting membrane potential) of adenosine on activity patterning in vivo, the simplest explanation of the observed effects is that endogenous adenosine acts principally on postspike potentials (most likely the DAP).

Given that antagonism of A1 receptors consistently increased burst duration and intraburst firing rate in phasically active vasopressin cells, it was surprising that it also induced phasic firing in continuously active vasopressin cells. However, several activity-dependent mechanisms provide negative feedback control of activity within bursts in phasic cells, and all might contribute to burst termination in some circumstances. Thus, as well as generating adenosine, electrical activity in phasic cells also induces secretion of vasopressin and dynorphin and production of nitric oxide, as well as inducing a slow hyperpolarization through activation of Ca2+-dependent K+ conductances (the AHP). Blockade of adenosine with CPT removes one mechanism potentially contributing to burst termination, but the resulting enhancement of activity would be expected to strengthen all other autoinhibitory activity-dependent mechanisms and thus, lead to burst termination.

In addition, adenosine has been reported to modulate the afferent synaptic inputs to the SON (12, 30); this involves an equipotent inhibition of GABA and glutamate release via activation of presynaptic A1 receptors. GABAergic and glutamatergic afferents are major connections on to magnocellular neurons accounting for 40% and 20% of synaptic inputs, respectively (13, 39). A CPT-induced increase in afferent synaptic noise at GABA and glutamatergic inputs would be expected to increase baseline noise and membrane potential fluctuations and so could account for the increased idfr in continuous cells. A lack of a similar effect in phasic cells was surprising given the importance of synaptic inputs in the maintenance of phasic activity in vivo. However, an effect on the idfr in these cells may be masked by the more prominent effects on intraburst firing mediated via changes in postspike potentials. Alternatively, phasic cells might have a different afferent GABA:glutamate input ratio that is enhanced proportionately by CPT. Whether the increased variability in continuous cell firing affects hormone output remains to be determined. However, we would expect that clustering of spikes during CPT treatment in oxytocin neurons and the adoption of a pseudophasic firing pattern in continuously active vasopressin cells might have a small enhancing effect on peptide output, because of frequency facilitation of secretion from the neurohypophysis without an effect on overall firing rate.

Although in vitro studies show an inhibitory effect of exogenous adenosine on SON neurons (32), the present study indicates that in vivo, there is a highly effective concentration of adenosine during normal activity, which would be expected to occlude potential effects induced by further increasing the extracellular concentration of adenosine via inhibition of reuptake or by mimicking adenosine actions with a receptor agonist. Indeed, preliminary studies support this argument (data not shown).

Adenosine also causes a dose-dependent inhibition of vasopressin and oxytocin depolarization-induced secretion from the isolated neurohypophysis (40). Thus the combined acute effects of adenosine at the cell body and neurohypophysis would appear to exert an important restraint of magnocellular neuron secretory activity, which could ultimately influence a number of fundamental physiological responses that depend upon oxytocin and vasopressin. Given the extensive distribution of adenosine in the brain, the results reported here could have important implications for the autocontrol of other neuronal systems, not just in terms of activity-dependent limitation of overall activity, but also for spike clustering, which may be of general importance in information processing and transfer.

GRANTS

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