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ADENOSINE PROMOTES BURST ACTIVITY IN GUINEA-PIG GENICULOCORTICAL NEURONES THROUGH TWO DIFFERENT IONIC MECHANISMS

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SUMMARY

1. The mechanisms of action of adenosine were examined in relay neurones of the dorsal lateral geniculate nucleus (LGND) using *in vitro* intracellular recording techniques in guinea-pig thalamic slices.

2. Adenosine hyperpolarized LGND relay neurones due to an increase in membrane potassium conductance. The K^+ currents generated by near maximal stimulation of adenosine and $GABA_B$ receptors were non-additive.

3. Blockage of membrane K^+ conductances by barium unmasked a second response to adenosine; an outward shift of the current *versus* voltage relationship negative to −65 mV associated with an increase in membrane input resistance. The β-adrenoceptor agonist isoprenaline elicited an inward current in the same voltage range, which was inhibited and replaced by an outward current during activation of adenosine receptors. The effects of adenosine were due to a decrease in amplitude and rate of rise of the hyperpolarization-activated cation current, I_h. Maximal reduction by 66% of I_h amplitude occurred near the range of half-activation.

4. Both responses to adenosine were mimicked by the selective A_1 receptor agonists N^6-cyclopentyladenosine or N^6-cyclohexyladenosine, and reversibly blocked by the selective A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX).

5. The decrease in I_h by adenosine may be mediated by an inhibition of adenylyl cyclase activity and hence a decrease in the intracellular level of cyclic AMP, since local application of the adenylyl cyclase inhibitor 2′,3′-dideoxyadenosine imitated the decrease in I_h. Local application of the adenylyl cyclase stimulant forskolin or 8-bromo-cyclic AMP resulted in an enhancement in I_h, and forskolin inhibited the action on I_h evoked by N^6-cyclopentyladenosine.

6. The adenosine-induced effects interacted with the intrinsic electrophysiological properties of LGND neurones in that (i) the hyperpolarization due to an increase in K^+ conductance inhibited single spike firing and promoted calcium-mediated burst discharges, and (ii) the decrease in I_h inhibited the dampening effect on Ca^{2+}-mediated rebound activity of β-adrenergic receptor stimulation.

7. It is suggested that during increased levels of extracellular adenosine the

response of LGND relay neurones to activating brainstem influences will be depressed, and a pattern of Ca^{2+}-mediated burst firing will be favoured.

INTRODUCTION

In the mammalian central nervous system, thalamic neurones are capable of gating the flow of sensory information from the periphery to the cortex depending upon the behavioural state of the animal (for review see Steriade & Llinás, 1988; Steriade, Jones & Llinás, 1990*a*). This gating function seems to be based upon intrinsic membrane properties of the thalamic neurones, properties of the local synaptic network, and the level of activity of extrathalamic input systems. Of particular interest here, thalamocortical relay neurones can generate action potentials in basically two different modes, which prevail during different functional states of the brain (McCarley, Benoit & Barrionuevo, 1983; Steriade & Deschênes, 1984; Steriade & Llinás, 1988). During certain stages of slow-wave sleep or drowsiness, the neurones generate stereotyped bursts of fast spikes, which are triggered by a calcium current with low threshold of activation, and which can occur in a rhythmic or non-rhythmic fashion (Jahnsen & Llinás, 1984*a*, *b*; Steriade & Deschênes, 1984; Steriade & Llinás, 1988; Steriade *et al.* 1990*a*). During periods of arousal or increased alertness, the neurones are relatively depolarized (Hirsch, Fourment & Marc, 1983) and the Ca^{2+} current is inactivated (Jahnsen & Llinás, 1984*a*, *b*; Steriade & Deschênes, 1984). During this state, the neurones generate sequences of single, sodium/potassium-mediated action potentials, which occur relatively independently of each other and the frequency of which depends upon the quality and strength of incoming stimuli (Steriade & Llinás, 1988; McCormick & Feeser, 1990). These state-dependent modes of thalamic activity are controlled to an important degree by ascending inputs from the upper brainstem core, using acetylcholine, noradrenaline, and serotonin as main neurotransmitters (for review see McCormick, 1989; Steriade & McCarley, 1990). Investigations both *in vivo* and *in vitro* have indicated that the ascending brainstem system is capable in thalamocortical neurones of dampening rhythmic or non-rhythmic burst firing, and of inducing a depolarization of the membrane into a mode of tonic repetitive single spike firing (McCormick, 1989; Steriade *et al.* 1990*a*). The brainstem influences thereby create a state of activity which is conducive to wakefulness.

While activity of the ascending brainstem system is generally assumed to be important for maintaining the waking state (Steriade & McCarley, 1990), the basic metabolic state of thalamocortical relay neurones may limit their responsiveness to activating brainstem influences. Of particular interest here is the ATP constituent adenosine which has been proposed to modulate neuronal activity depending upon the intrinsic availability of high energy metabolites (Dunwiddie, 1985; Snyder, 1985). Adenosine is endogenously present in the cerebrospinal fluid in concentrations between 1 and 10 μM under normal conditions (Dunwiddie, 1985); high affinity binding sites for adenosine analogues have been demonstrated to exist (reviewed by Williams, 1987; Silinsky, 1989); there is evidence for enzymatic systems of degradation and uptake of adenosine (Wu & Phillis, 1984); adenosine is released in a stimulation-dependent way from axon terminals (Schubert, Lee, West, Deadwyler & Lynch, 1976), and adenosine concentrations have been found to rise substantially

during increased metabolic activity in the brain (Dunwiddie, 1985). In different neuronal systems, application of adenosine exerts an inhibitory tone through depression of both pre- and postsynaptic activity (see Nicoll, Malenka & Kauer, 1990). The actions of adenosine may be particularly important for modulating the sleep–waking cycle (Radulovacki, Virus, Djuricic-Nedelson & Green, 1984).

In the thalamus, the effects of adenosine are largely unknown. A short microionophoretic study performed in the thalamus *in vivo* demonstrated an inhibition of single spike activity by adenosine (Kostopoulos & Phillis, 1977), whereas postsynaptic potentials elicited in the lateral geniculate nucleus *in vitro* through stimulation of the optic tract were not influenced by adenosine (Okada & Saito, 1979). On the other hand, quantitative receptor autoradiography demonstrated the existence of adenosine receptors throughout the thalamus (Lee & Reddington, 1986), with high concentrations occurring in the dorsal lateral geniculate nucleus (LGND), the main thalamic station of the visual pathway. These receptors are probably not associated with terminals of the retinogeniculate or corticothalamic pathway (Goodman, Kuhar, Hester & Snyder, 1983).

The present study evaluates the actions of adenosine on cortically projecting relay neurones in the guinea-pig LGND maintained *in vitro*. The results indicate that adenosine evokes two prominent effects; an increase in membrane conductance for K^+ ions, and a decrease of the hyperpolarization-activated cation current (I_h), both of which can promote a state of burst activity in LGND relay neurones.

METHODS

Experiments were performed in slices of the LGN prepared from male or female guinea-pigs (200–350 g) as described previously (Pape & McCormick, 1989; McCormick & Pape, 1990*a*, *b*). During deep anaesthesia (pentobarbitone I.P., 40 mg/kg) the animals were decapitated and a block of tissue containing the LGN was rapidly removed. Slices were prepared in physiological saline (at a temperature of 5 °C) as 400 μm thick coronal sections on a vibratome (Ted Pella, Model 1000, Redding, USA) and transferred to an interface-type recording chamber (Fine Science Tools, North Vancouver, Canada). Slices were maintained at 36±1 °C and continuously superfused with oxygenated saline. Epi-illumination of the coronal slices readily revealed the dorsal and ventral part of the LGN. The composition of the saline was (in mM): NaCl, 126; KCl, 2·5; $MgSO_4$, 2; $NaHCO_3$, 26; NaH_2PO_4, 1·25; $CaCl_2$, 2; dextrose, 10; buffered to a final pH of 7·4 through continuous perfusion of 95% O_2–5% CO_2. Slices were allowed to equilibrate for at least 2 h before recording commenced.

Thin-walled glass microelectrodes (World Precision Instruments, New Haven, USA, TW-100F) for intracellular recordings were prepared on a Flaming-Brown miropipette puller (Model P-87, Sutter, San Rafael, USA). Electrodes were back-filled with 4 M-potassium acetate. Final resistance ranged between 35 and 55 MΩ.

Intracellular recordings under current-clamp or voltage-clamp conditions were controlled through an Axoclamp 2A amplifier (Axon Instruments, Foster City, USA). During current-clamp recordings, the bridge balance was continuously monitored. During discontinuous current-clamp or voltage-clamp recordings, the headstage output was continuously monitored to ensure adequate settling time. Sampling frequences were between 3 and 5 kHz, and the amplifier gain was between 0·7 and 1 nA/mV. Current *versus* voltage (*I–V*) relationships were obtained under voltage clamp by steadily hyperpolarizing the neurones (voltage ramp of 1–8 s duration) from a holding potential around −50 mV to a final potential around −110 mV. Voltage ramps were repeated every 10–16 s and individual current traces (two to eight) were averaged to reduce noise. Voltage-clamp experiments were governed by pClamp software (Axon Instruments) operating via a LabMaster DMA interface (Model TL-1-125, Axon Istruments) on an IBM AT computer. Data were collected on-line with the computer or digitized (NeuroCorder DR-384; Neurodata, New York) and stored on videotape (Videocassette Recorder E70, Mitsubishi) for later analysis. Neurones were recorded

in the dorsal part of the LGN, and only those having stable membrane potentials negative to −55 mV, resting input resistances above 35 MΩ and action potentials overshooting 0 mV were collected for analysis. At the end of each experiment, possible changes in electrode tip potential were controlled by measuring the DC offset of the electrode in the bathing medium, and the value of the measured membrane potential was corrected accordingly. Changes in electrode tip potential were usually below 4 mV. Synaptic potentials in the LGND were evoked by electrical microstimulation with a tungsten bipolar electrode (20–100 μs, 0·1–2 mA), which was placed in the optic tract or within the LGND itself.

Drugs were applied to LGND neurones either in the bathing medium or locally to the exposed surface of the slice through a broken micropipette (2–5 μm tip diameter) by applying pressure pulses of nitrogen (5–20 ms, 400 kPa) to the pipette. Typically, 5–20 pl of the drug were applied to the slice within 50–100 μm of the entry point of the recording electrode. When barium was introduced in the perfusion medium, $MgCl_2$ was substituted for $MgSO_4$, and NaCl for NaH_2PO_4 to avoid precipitation. During perfusion of barium or 4-aminopyridine, tetrodotoxin (TTX, 1–1·5 μM) was added to the medium to prevent excessive synaptic activity. Changing the extracellular concentration of KCl was achieved through equimolar substitution of NaCl.

All substances were obtained from Sigma, except 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 5′-(*N*-cyclopropyl)-carboxamidoadenosine (CPCA), N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine (DPMA), 5′-*N*-ethylcarboxamidoadenosine (NECA), which were obtained from Research Biochemicals Inc. The gift of CGS 21680 from Ciba-Geigy, USA, is gratefully acknowledged. Drugs were dissolved in the bathing medium immediately before use. DPCPX was dissolved in dimethylsulphoxide (DMSO) and stored as a 10 mM stock solution at 4 °C. CGS 21680, DPMA and forskolin were dissolved in DMSO and prepared as a 250 μM or 500 μM stock solution. NECA was dissolved in 0·1 N-HCl and prepared as a 1 mM stock solution, N^6-cyclopentyladenosine (CPA) and N^6-cyclohexyladenosine (CHA) were dissolved in ethanol and prepared as 500 μM stock solutions. All drugs were diluted in the bathing solution to final concentration just before use. The final concentration of ethanol was below 0·2% (local application) and 0·005% (bath application); DMSO was below 0·03% during local application. L-Ascorbic acid was sometimes added in equimolar concentration to isoprenaline to prevent rapid oxidation. Responses to isoprenaline were identical with and without ascorbic acid.

Averaged data are presented as means ± standard deviation.

RESULTS

Stable intracellular recordings were obtained from 125 LGND neurones. The basic electrophysiological properties of these neurones (i.e. rebound burst discharges upon repolarization after hyperpolarization, strong inward rectification in the hyperpolarizing direction, and delayed onset of spike firing upon depolarizing stimuli) were typical of those described for thalamocortical relay neurones (Jahnsen & Llinás, 1984*a*; Crunelli, Leresche & Parnavelas, 1987; McCormick & Pape, 1988). A representative sample of forty LGND neurones possessed an average resting membrane potential of −63 ± 3 mV, an apparent resting input resistance of 51 ± 13 MΩ, and an action potential amplitude of 84 ± 7 mV.

Local application of adenosine (2–5 mM) to LGND neurones resulted in a hyperpolarization from normal resting potential in all tested neurones ($n = 55$; Fig. 1), which was associated with a peak increase in apparent input conductance of 5–15 nS ($n = 38$). This response persisted during blocked synaptic transmission in a bathing solution containing TTX (1–1·5 μM; $n = 4$) or a low Ca^{2+} (0·5 mM) and high Mg^{2+} (4 mM) concentration ($n = 2$), indicating a direct postsynaptic effect of the recorded neurone (not shown).

Ionic mechanisms of the adenosine-induced hyperpolarization

The current evoked by adenosine (as measured under voltage-clamp conditions by applying voltage ramps of 1 s duration from about −55 to −110 mV) reversed polarity at −99±5 mV ($n = 18$) in bathing solutions containing 2·5 mM-K^+ (Fig.

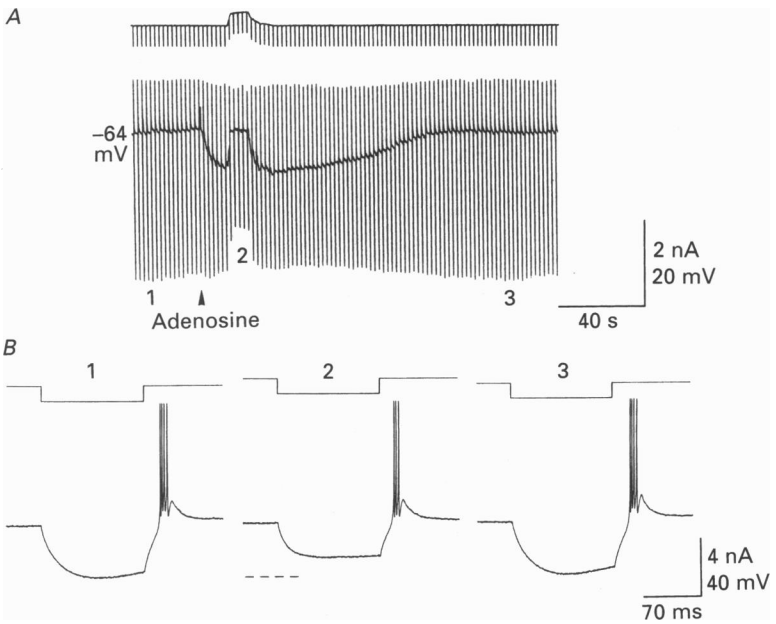

Fig. 1. Typical response of an LGND relay neurone to adenosine. *A*, local application of adenosine (3 mM) results in a membrane hyperpolarization from normal resting potential (−64 mV, as indicated). Injection of hyperpolarizing constant current pulses (−0·5 nA, 120 ms, 0·5 Hz) and resulting deflections in membrane potential indicate the apparent input resistance. Compensation of the adenosine-induced hyperpolarization with intracellular injection of outward current reveals a decrease in input resistance associated with the response (2). *B*, responses of the neurone to the constant current pulse before (1), during (2), and following recovery (3) from adenosine are expanded for comparison. Dashed line in (2) indicates potential deflection through the current pulse before application of adenosine. Note rebound burst activity, which is due to activation of the low-threshold Ca^{2+} spike, triggering a set of fast Na^+/K^+-mediated action potentials. Upper trace is membrane current; lower trace is membrane potential. Spikes in *A* are truncated.

2*A*). Elevating the extracellular K^+ concentration to 7·5 mM shifted the reversal potential to −77±3 mV ($n = 4$; Fig. 2*B*), indicating K^+ ions as the main charge carriers of the adenosine-induced current. The underlying conductance was not obviously voltage dependent in the range −55 to −110 mV. In these experiments, the action of adenosine on the slow, hyperpolarization-activated cation current, I_h (see below), was minimized through two different methods: (i) the duration of the voltage ramp was set to 1 s, during which I_h was only partially activated, or (ii) I_h was blocked by local application of low concentrations of caesium (5–20 mM; cf. McCormick & Pape, 1990*a*).

In hippocampal pyramidal neurones (Andrade, Malenka & Nicoll, 1986; Zgombick,

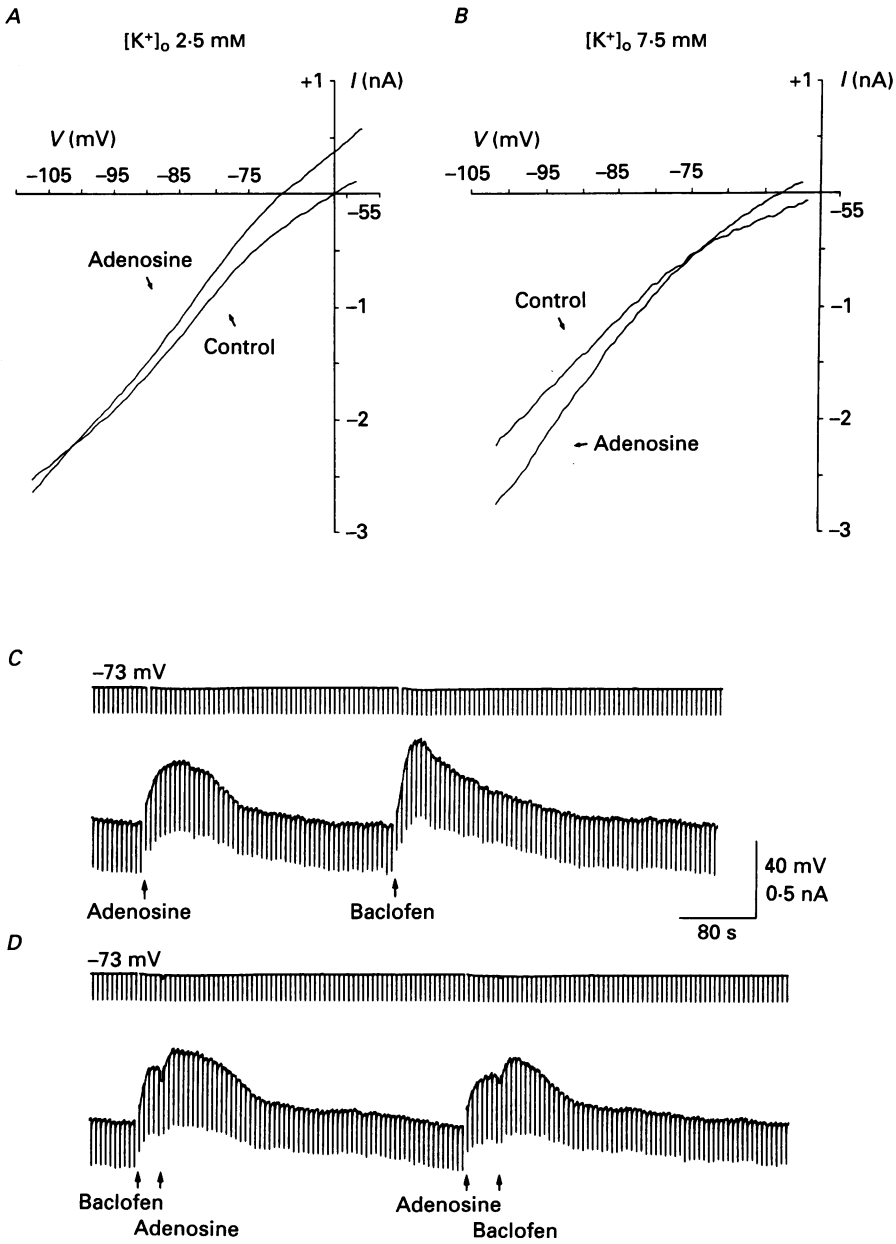

Fig. 2. Adenosine increases a K^+ conductance. *A* and *B*, current *versus* voltage (*I–V*) relationships obtained under voltage-clamp conditions by applying a hyperpolarizing voltage ramp of 1 s duration from −58 mV to approximately −110 mV before (control) and during action of adenosine (3 mM, local). The reversal potential of the adenosine-induced current is shifted from −102 to −73 mV by elevating the K^+ concentration of the bathing medium from 2·5 mM (*A*) to 7·5 mM (*B*). Note lack of obvious voltage dependence of the adenosine-evoked effect. *C* and *D*, non-additivity of adenosine and baclofen action. Local application of either adenosine (3 mM) or the $GABA_B$ agonist baclofen (100 μM) evokes an outward current associated with an increase in conductance from a holding potential of −73 mV (*C*), while a near-maximal application of baclofen

Beck, Mahle, Craddock-Royal & Maayani, 1989) and neocortical neurones (McCormick & Williamson, 1989), adenosine receptors have been proposed to share the same K^+ conductance with $GABA_B$ and 5-HT_{1A} receptors. In LGND neurones, the K^+ current generated by a near-maximal application of adenosine was reduced by $82 \pm 13\%$ ($n = 4$) during the peak K^+ current elicited by the $GABA_B$ agonist baclofen (Fig. 2*C* and *D*). Similarly, a near-maximal activation of adenosine receptors reduced the response to baclofen by $76 \pm 10\%$ ($n = 4$). This non-additivity suggests convergence of the two receptor types onto one postsynaptic mechanism, presumably the same set of membrane K^+ channels (cf. Nicoll, 1988).

Pharmacology of the hyperpolarizing response

Adenosine receptors can be roughly subdivided into A_1 and A_2 receptor subtypes based upon the potency of selective agonists and antagonists (see Williams, 1987). In LGND neurones, local application (1–10 μM) of the highly selective A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine, DPCPX (Lohse, Klotz, Lindenborn-Fotinos, Reddington, Schwabe & Olsson, 1987) readily and reversibly blocked the hyperpolarizing response to adenosine in all neurones that were tested ($n = 4$; Fig. 3*A–C*). Local application of the selective A_1 agonists (Bruns, Daly & Snyder, 1980; Williams, Braunwalder & Erickson, 1986) N^6-cyclohexyladenosine (CHA, 50–300 μM; $n = 9$) and N^6-cyclopentyladenosine (CPA, 50–300 μM; $n = 12$) imitated the adenosine-induced hyperpolarization and increase in K^+ conductance (Fig. 3*D*). Compared with adenosine, the time to peak and recovery period of responses induced by CHA or CPA were slow, presumably due to physical properties of the agonists (such as lipid solubility; Dunwiddie, 1985) and/or re-uptake or metabolism of adenosine (Wu & Phillis, 1984). Addition of CPA (0·5–1 μM) to the bathing medium resulted in a substantial hyperpolarizing shift of the resting membrane potential (-70 ± 3 mV, $n = 5$) and a decrease in resting input resistance (38 ± 11 MΩ) due to an increase in membrane K^+ conductance, which was inhibited by local application of the adenosine antagonist theophylline (1–5 mM). Local application of potent agonists for the A_2 site (Daly, 1982; Bruns, Lu & Pugsley, 1986; Williams, 1987), namely 5′-*N*-ethylcarboxamidoadenosine (NECA, 150 μM; $n = 2$) and 5′-(*N*-cyclopropyl)-carboxamidoadenosine (CPCA, 20–300 μM; $n = 9$), also evoked an increase in K^+ conductance (data not shown). Application of the selective A_1 antagonist DPCPX (1–10 μM, local) completely blocked these responses in all neurones tested ($n = 5$), indicating an involvement of A_1, rather than A_2 receptors. This notion is further supported by the finding that local application of the more selective A_2 agonists (Bridges, Bruns, Ortwine, Priebe, Szotek & Trivedi, 1988; Hutchison, Webb, Oei, Ghai, Zimmermann & Williams, 1989) N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine (DPMA, 5–20 μM; $n = 2$) or CGS 21680 (up to 200 μM; $n = 2$) did not imitate the adenosine-induced increase in K^+ conductance (data not shown).

These results indicate that in LGND neurones adenosine increases a membrane K^+ conductance through A_1 receptors. Application of the adenosine antagonists DPCPX

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nearly occludes the adenosine-induced current, and vice versa (*D*). Recordings are from two different LGND neurones (*A*, *B* and *C*, *D*). Caesium (15 mM) was locally applied to the neurone in *A* and *B* to block I_h.

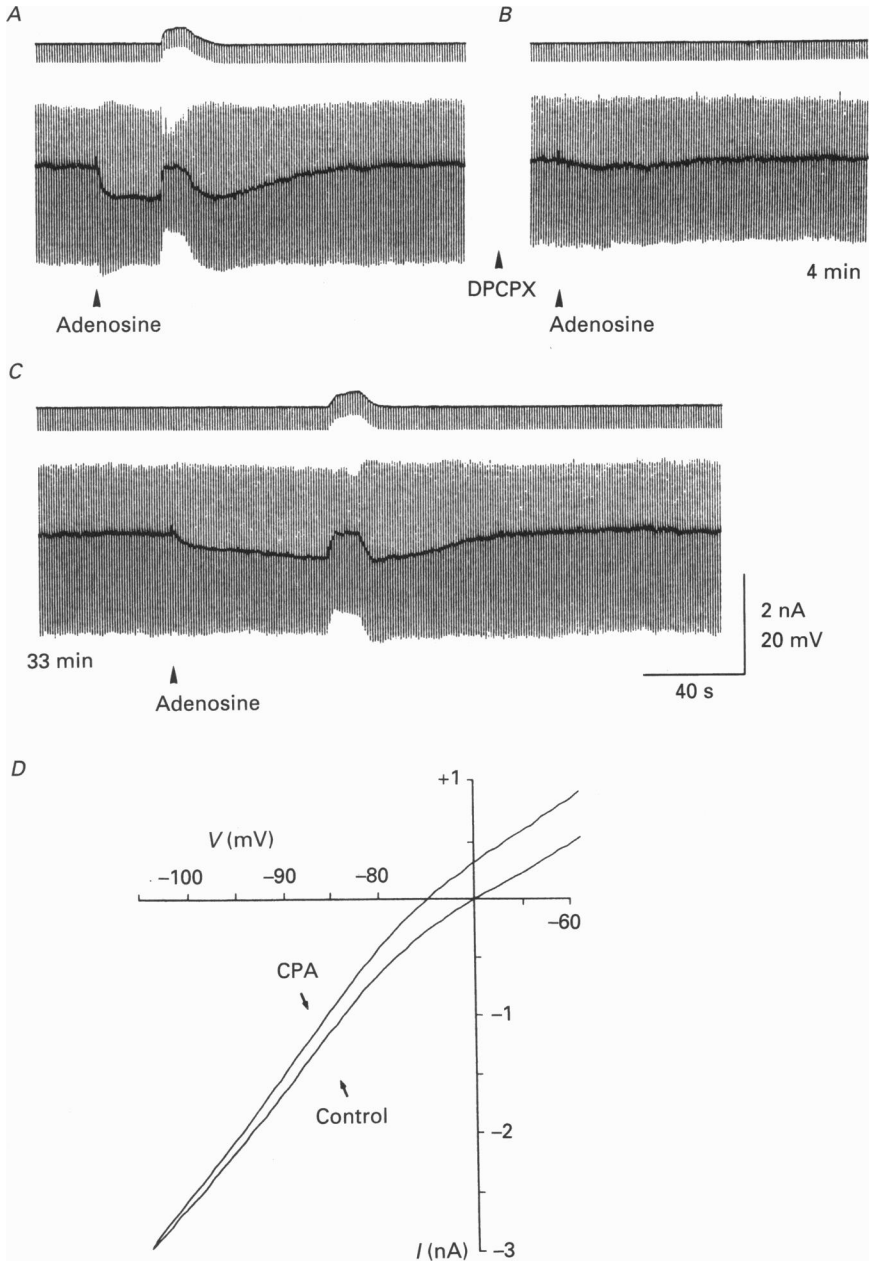

Fig. 3. Adenosine-induced increase in K^+ conductance is mediated via A_1 receptors. *A–C*, the typical membrane hyperpolarization and associated decrease in apparent input resistance evoked by adenosine (5 mM, local; *A*) are greatly reduced following local application of the selective A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine, DPCPX (5 μM; *B*), and partially recover from block within 33 min (*C*). Numbers near traces indicate time in minutes after application of DPCPX. Upper trace is current, lower trace is membrane potential. *D*, *I–V*-relationship obtained under voltage-clamp conditions. Local application of the selective A_1 receptor agonist N^6-cyclopentyladenosine

(1–10 μM, local) or theophylline (up to 5 mM, local) had no effect of their own on membrane potential or input resistance, implying no detectable activation of K^+ conductance by adenosine present in the LGND slice preparation.

Changes in neuronal activity through adenosine action

Thalamocortical neurones possess two basic modes of action potential generation, depending upon the value of the prevailing membrane potential (Jahnsen & Llinás, 1984*a*, *b*). Na^+/K^+-mediated single spike firing occurs at slightly depolarized membrane potentials, and burst activity, mediated by a Ca^{2+} spike with low threshold of activation, occurs in the hyperpolarized range of voltage. As reported previously (Jahnsen & Llinás, 1984*a*; McCormick & Pape, 1988), injection of a small depolarizing current pulse into LGND neurones at normal resting potential elicited a sequence of tonic repetitive firing of Na^+/K^+-mediated action potentials, which was typically delayed in onset (Fig. 4*B*1). Local application of adenosine (2–5 mM) induced a membrane hyperpolarization from rest, due to the increase in K^+ conductance, and thereby inhibited single spike firing and moved the membrane potential into a range where the low-threshold Ca^{2+} current is partly de-inactivated. Now the depolarizing current pulse elicited a typical burst response, consisting of the low-threshold Ca^{2+} spike with a set of fast Na^+/K^+ action potentials riding its crest (Fig. 4*B*2). This burst response during the adenosine-induced hyperpolarization was similar to that elicited when the neurones were hyperpolarized to the same potentials by intracellular injection of direct current following recovery from the adenosine response. This result indicates that the ability of adenosine to promote burst firing is due largely to the associated change in membrane potential. In some neurones, the burst response during the adenosine-induced hyperpolarization contained one to two fast action potentials less than that during the direct current-induced hyperpolarization, most probably due to the increase in membrane conductance associated with the adenosine response.

Adenosine increases the apparent input resistance via A_1 receptors

During the decaying phase and immediately following the typical hyperpolarizing response to adenosine, the apparent input resistance of the neurones appeared to be larger compared with that before application of adenosine (Fig. 5*A* and *B*), suggesting a response different from the K^+ conductance increase. Indeed, blockage of K^+ channels through bath application of barium (600–800 μM) blocked the adenosine-induced K^+ conductance increase (see also Gerber, Greene, Haas & Stevens, 1989) in 92% of the neurones ($n = 39$) and unmasked an adenosine-evoked, slow increase in apparent input resistance, associated with a 1–2 mV hyperpolarization from resting potential (Fig. 5*C* and *D*). Unlike in hippocampal CA1 pyramidal neurones (Gerber *et al.* 1989), addition of the K^+ channel blocker 4-aminopyridine (4-AP, up to 10 mM) did not substantially affect the K^+ conductance increase mediated by adenosine.

In the following experiments, after responses to adenosine in the control solution

(CPA, 100 μM) elicits an outward current which reverses near the presumed K^+ equilibrium potential. Recordings are from two different LGND relay neurones (*A–C* and *D*).

had been recorded, Ba^{2+} (600–800 μM) was added to the bathing solution to isolate the adenosine-mediated increase in apparent input resistance. Tetrodotoxin (TTX, 1–1·5 μM) was included in the solution to prevent synaptic activity. In the Ba^{2+}-containing solution, resting membrane potentials were slightly shifted in the

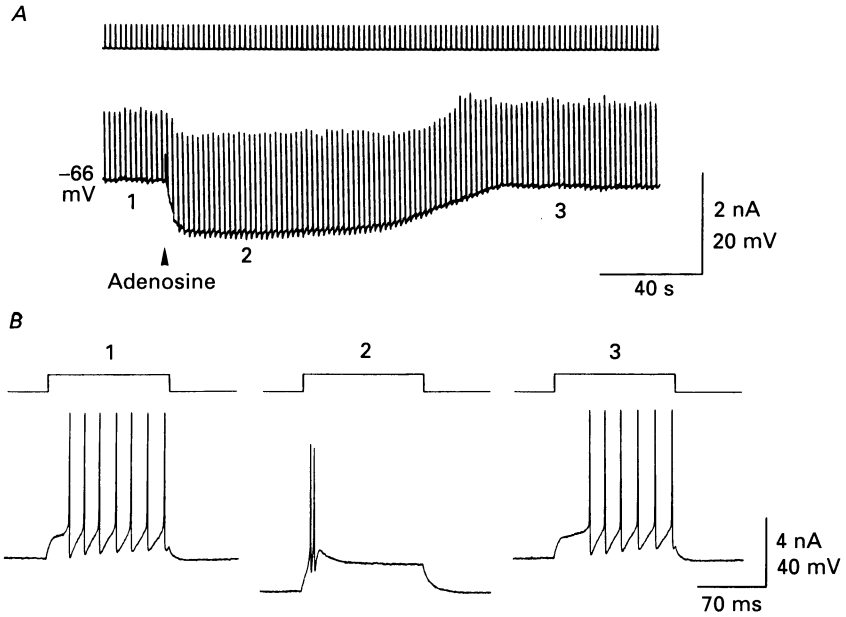

Fig. 4. Adenosine inhibits single spike firing and promotes burst activity in an LGND neurone. *A*, application of adenosine (3 mM; local) elicits the typical hyperpolarizing response. Examples of responses to small depolarizing current pulses (+0·5 nA, 120 ms, 0·5 Hz) are shown at a faster time scale in *B*. At normal resting potential (−66 mV), the current pulse elicits a series of tonic firing of Na^+/K^+-mediated action potentials, which is delayed in onset (1); during the adenosine-induced hyperpolarization, the low-threshold Ca^{2+} spike is partly de-inactivated and the current pulse evokes a typical burst response consisting of the low-threshold Ca^{2+} spike triggering two fast action potentials. Recovery is shown in (3).

depolarizing direction, and resting input conductances were decreased. Under these conditions, an increase in membrane resistance was elicited either by adenosine (2–5 mM, local application) or by A_1 selective agonists (CPA, CHA; 100–300 μM, local; $n = 12$) in 89% of the tested neurones ($n = 36$), whereas A_2 selective agonists (DPMA, 5–20 μM; CGS 21680, up to 200 μM; local) did not exert a substantial effect. The notion that A_1 receptors are involved in the mediation of the decrease in apparent input conductance was further supported by the finding that this response was readily and reversibly abolished by the highly selective A_1 antagonist DPCPX (5–10 μM; local; $n = 4$; not shown).

Activation of A_1 receptors decreases I_h

Adjusting the membrane potential to different values through intracellular injection of direct current revealed a striking voltage dependence of the adenosine-induced response. Application of adenosine to slightly depolarized neurones (e.g.

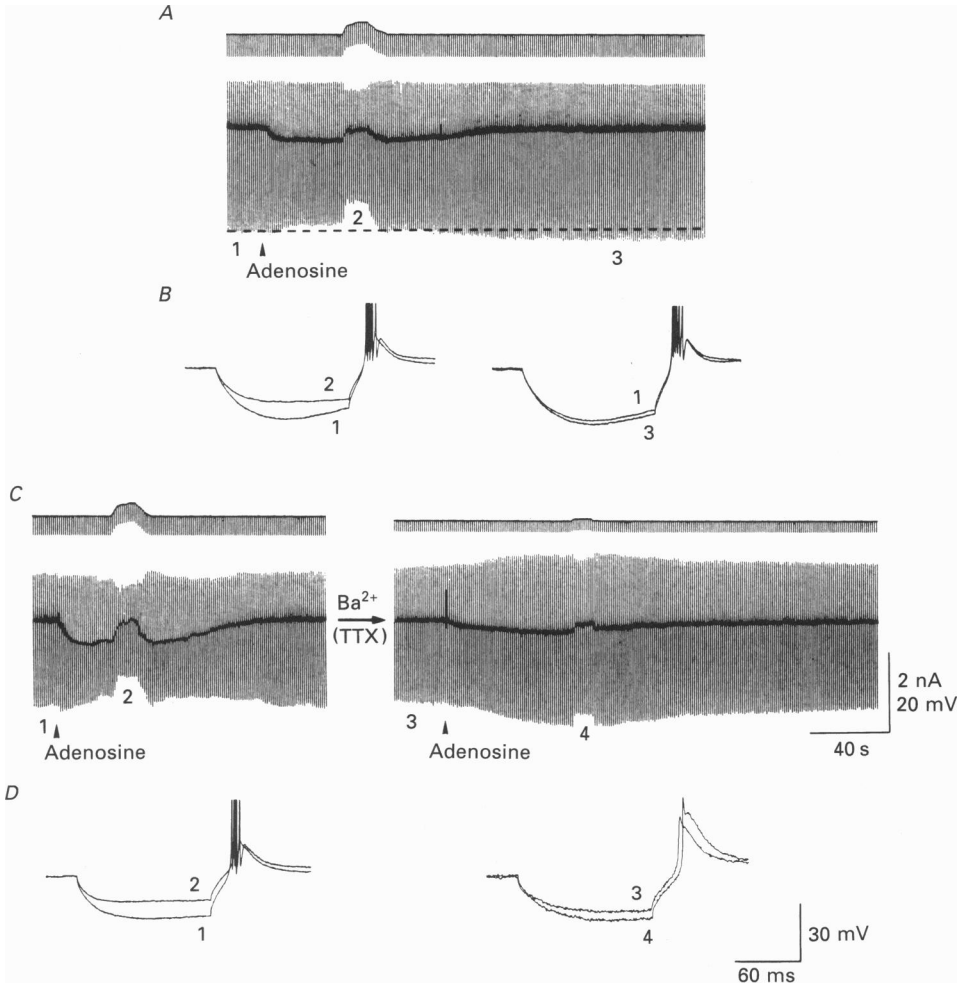

Fig. 5. Blockage of K^+ conductances unmasks an adenosine-evoked increase in apparent input resistance. *A*, local application of adenosine (3 mM) elicits a membrane hyperpolarization and decrease in apparent input resistance, followed by a small increase in apparent input resistance. Dashed line indicates level of voltage deflection induced by hyperpolarizing current pulses before application of adenosine. Responses to current pulses before (1) and during (2 and 3) adenosine are expanded for comparison in *B*. *C*, addition of barium (Ba^{2+}, 700 μM) to the bathing medium blocks the adenosine-evoked hyperpolarization and resistance decrease, and unmasks a small hyperpolarization of longer duration associated with an increase in input resistance, as a response to adenosine. Examples of responses to current pulses in *D* demonstrate decreased (*D*2 *versus* *D*1), and increased input resistance (*D*4 *versus* *D*3), associated with the adenosine response before and during addition of Ba^{2+}, respectively. Note that the increase in the depolarizing response to the current pulse during adenosine (*D*3 to *D*4) results from an increased rebound Ca^{2+} spike, which is increasingly de-inactivated through the increase in the preceding hyperpolarizing response to the current pulse. Tetrodotoxin (TTX, 1 μM) was added to block synaptic activity (note blocked Na^+/K^+-mediated action potentials in *D*3 and *D*4). Recordings are from two different LGND relay neurones (*A*, *B*, and *C*, *D*). Upper trace is current, lower trace is membrane potential. Scale bars in *C* also for *A*; scale bars in *D* also for *B*. Fast action potentials are truncated.

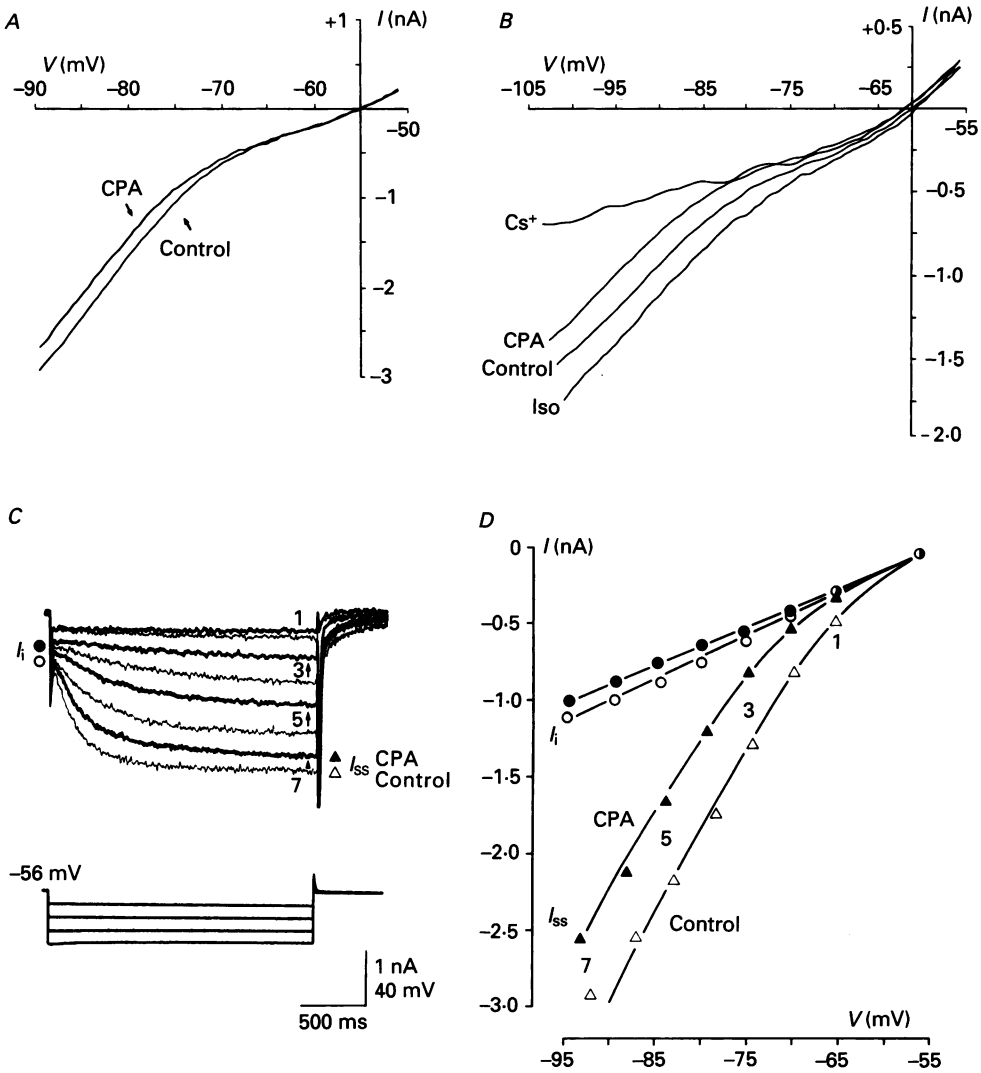

Fig. 6. Activation of A_1 receptors decreases I_h. *A* and *B*, *I–V* relationships measured under voltage-clamp conditions (duration of the voltage ramp was increased to 6 s to ensure near steady-state activation of I_h). *A*, local application of the A_1 selective agonist CPA (300 μM) results in an outward shift negative to −65 mV. *B*, application of the β-adrenergic agonist isoprenaline (Iso, 100 μM; local) induces an inward shift negative to −65 mV; application of CPA (300 μM, local) inhibits the β-adrenergic response and induces an outward shift in the same voltage range. Caesium (Cs^+, 10 mM; local) blocks inward rectification. *C* and *D* incremental voltage steps from a holding potential of −56 mV elicit the time- and voltage-dependent inward current, I_h. The larger current in each pair in *C* (see arrow) was recorded under control conditions, the smaller current was elicited by the same voltage step during action of CPA (300 μM, local). CPA reduces the slow inward current (I_{ss}) with little effect on instantaneous current (I_i). *D*, *I–V* relationship from experiment in *A*, showing the complete family of voltage steps and currents. I_i is plotted as circles, I_{ss} is plotted as triangles, the difference represents I_h at a given membrane potential. Open symbols represent currents obtained under control conditions, closed symbols are currents during action of CPA. Numbers near symbols indicate

−54 mV) did not result in a consistent effect, while at increasingly negative membrane potentials adenosine increasingly elicited the slow increase in apparent input resistance and small hyperpolarization. This apparent voltage dependence was further investigated under voltage-clamp conditions. As mentioned already, the selective A_1 agonist CPA elicited responses that mimicked those to adenosine, but that were substantially longer in duration. Thus, to ensure adequate analysis over a longer period of time, CPA was used in most of the voltage-clamp experiments. *I–V* relationships obtained by continuously hyperpolarizing the membrane potential from about −50 to −100 mV over a period of 5–8 s before and during application of CPA (100–300 μM, local) revealed that activation of A_1 receptors induced a highly voltage-dependent outward shift in the hyperpolarizing range. The membrane potential where the A_1 receptor-mediated outward shift became first apparent was -65 ± 5 mV ($n = 8$; Fig. 6*A*).

The strong dependence on hyperpolarizing voltage of the A_1 receptor-activated current suggested that it was mediated through modulation of the hyperpolarization-activated cation current, I_h, which possesses a very similar voltage dependence (McCormick & Pape, 1990*a*). Indeed, increasing I_h through activation of β-adrenergic receptors (local application of isoprenaline, 50–300 μM; Pape & McCormick, 1989) elicited an inward current in the hyperpolarizing range of voltage. Local application of CPA (100–300 μM) inhibited this increase in I_h and elicited an outward current in the same voltage range, indicating a reduction of I_h to below control values (Fig. 6*B*). Furthermore, activation of β-adrenergic receptors through isoprenaline inhibited the response to A_1 receptor stimulation. Blockage of I_h through local application of caesium (5–20 mM; McCormick & Pape, 1990*a*) abolished the inwardly rectifying membrane responses. Next, I_h was elicited by hyperpolarizing voltage steps of 2 s duration to between −60 and −100 mV from holding potentials around −55 mV. Local application of CPA (100–300 μM) resulted in a marked decrease in amplitude of I_h with very little, if any, change in instantaneous conductance at the beginning of the hyperpolarizing voltage step ($n = 6$; Fig. 6*C* and *D*). Furthermore, the time constant of activation of I_h was substantially increased during stimulation of A_1 receptors. Previous increase in I_h by addition of isoprenaline (0·5–1 μM) to the bathing medium resulted in a larger net effect of A_1 receptor activation ($n = 3$; compare Fig. 6*B*). I_h maximally decreased after CPA application by 66 ± 5% near its half-activation range (−74 mV; cf. McCormick & Pape, 1990*a*), while the decrease was 13 ± 5% near the range of full activation (−95 mV). Application of adenosine elicited a similar decrease in I_h ($n = 7$), though the effects were typically smaller in amplitude when compared with those of CPA (not shown).

Decrease in I_h by adenosine may involve inhibition of adenylyl cyclase activity

Activation of A_1 binding sites has been shown to inhibit adenylyl cyclase activity and hence to reduce the intracellular level of cyclic AMP in different preparations (Van Calker, Müller & Hamprecht, 1979). Since the adenylyl cyclase–cyclic AMP

examples shown in *A*. Ba^{2+} (600–800 μM) and TTX (1 μM) were included in the bathing solution to block K^+ and Na^+ conductances, and in addition in *C* and *D*, isoprenaline (1 μM) was added.

system has been shown to be involved in β-adrenergic or serotonergic receptor-mediated enhancement of I_h in LGND neurones (Pape & McCormick, 1989; McCormick & Pape, 1990*b*), we investigated the possible contribution of adenylyl cyclase activity to the decrease in I_h after A_1 receptor activation.

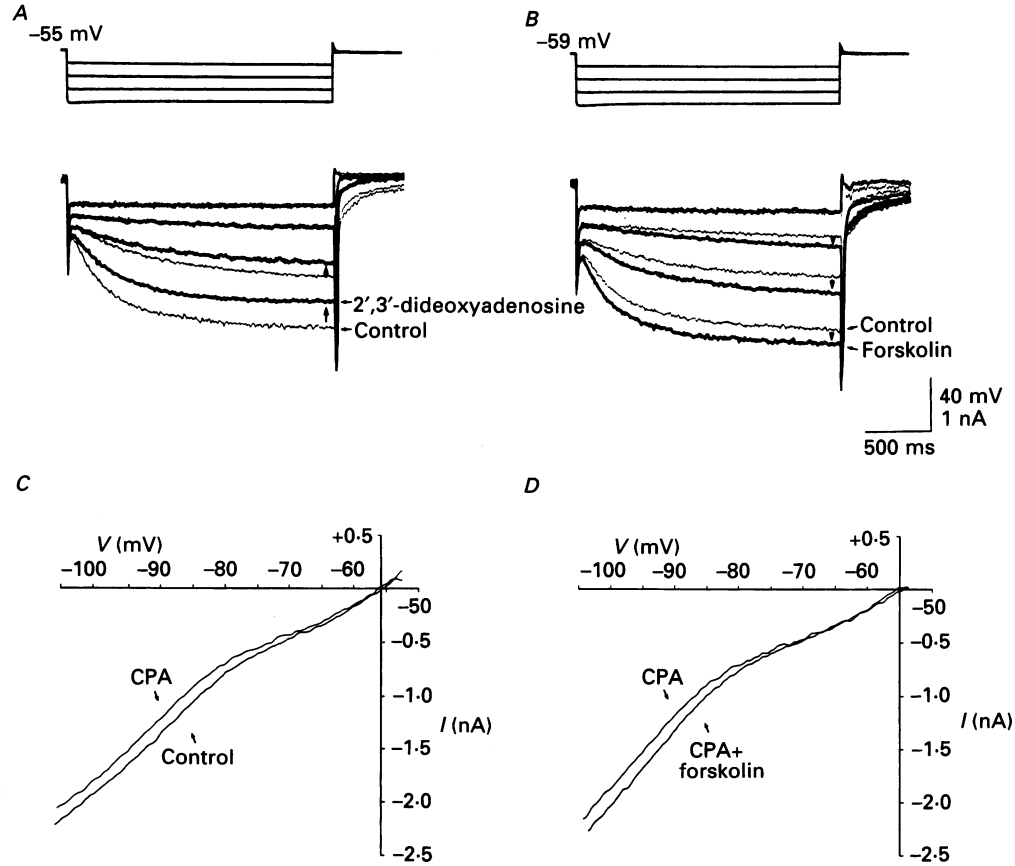

Fig. 7. Modulation of I_h through altered adenylyl cyclase activity. *A*, local application of the adenylyl cyclase inhibitor 2′,3′-dideoxyadenosine (100 μM) results in a decrease in I_h with no significant change in instantaneous current. *B*, local application of the adenylyl cyclase stimulant forskolin (50 μM) increases I_h with no change in instantaneous current. Upper trace is membrane potential (with holding potentials indicated), bottom traces are membrane current. The thin trace of each pair represents current under control conditions, the thick trace is current obtained during action of adenylyl cyclase modulators (see arrows). Bathing medium contained no Ba^{2+}, no TTX, and no isoprenaline. *C* and *D*, *I–V* relationships under voltage clamp. CPA (300 μM, local) induced decrease in I_h results in an outward shift negative to −65 mV (*C*), which is blocked by addition of forskolin (25 μM, local; *D*). Bathing medium contained Ba^{2+} (800 μM), TTX (1 μM).

The compound 2′,3′-dideoxyadenosine acts with high specificity at the catalytic subunit of adenylyl cyclase from rat brain and is inhibitory to this enzyme (Londos & Wolff, 1977; Johnson, Yeung, Stübner, Bushfield & Shoshani, 1989). In the LGND, local application of 2′,3′-dideoxyadenosine (50–100 μM; $n = 3$) resulted in a

substantial decrease in I_h with no change in instantaneous current at the beginning of the hyperpolarizing voltage steps (Fig. 7*A*). By contrast, local application of the adenylyl cyclase stimulant, forskolin (25–50 μM, $n = 4$; Fig. 7*B*), or the membrane permeable cyclic AMP analogue, 8-bromo-cyclic AMP (500 μM; $n = 2$), enhanced I_h, as has been previously reported (McCormick & Pape, 1990*b*). Furthermore, the A_1 receptor-mediated decrease in I_h was antagonized by local application of forskolin (Fig. 7*C* and *D*). The protein kinase inhibitor H8 (300 μM, local application) did not exert a detectable effect on I_h ($n = 2$; not shown).

These data indicate that stimulation of A_1 receptors reduces I_h via inhibition of adenylyl cyclase activity and hence a decrease in intracellular level of cyclic AMP.

Possible functional implications of A_1-mediated decrease in I_h

I_h has been proposed to be particularly important during rhythmic burst activity of thalamocortical neurones (McCormick & Pape, 1990*a*), which occurs *in vivo* predominantly during certain periods of electroencephalographic synchronization, such as slow wave sleep and drowsiness (Steriade *et al.* 1990*a*). Oscillatory activity of thalamocortical relay neurones can occur as intrinsically generated 1–2 Hz burst activity or as spindle oscillations (Lamarre, Filion & Cordeau, 1971; Steriade & Deschênes, 1984; Leresche, Jassik-Gerschenfeld, Haby, Soltesz & Crunelli, 1990; McCormick & Pape, 1990*a*). Spindle oscillation consists of groups of discharges at 7–14 Hz and of one to several seconds duration, which periodically re-occur every 3–10 s (Steriade *et al.* 1990*a*). Spindling is based upon generation of rhythmic inhibitory postsynaptic potentials (IPSPs), mediated through reticular thalamic neurones containing γ-aminobutyric acid (Steriade & Deschênes, 1984). The IPSPs seem to provide enough membrane hyperpolarization to de-inactivate the low-threshold Ca^{2+} current, which during the repolarizing phase of the IPSP generates a Ca^{2+} spike, which in turn may reach threshold for generation of Na^+/K^+ action potentials. In this manner, spindle oscillation consists of the rhythmically re-occurring sequence of membrane hyperpolarization and rebound Ca^{2+}-mediated burst activity. One possible way to dampen this oscillatory activity is to prevent sufficient de-inactivation of the Ca^{2+} current to occur and thereby to prevent the generation of the rebound Ca^{2+} spike. For example, the *increase* in I_h, due to activation of β-adrenergic or serotonergic receptors, substantially reduces the amplitude and duration of strong membrane hyperpolarizations and increases the input conductance of the membrane, thereby probably providing an effective mechanism to dampen spindle oscillations in the thalamus (Pape & McCormick, 1989; McCormick & Pape, 1990*b*).

Adenosine, via the A_1 receptor-mediated *decrease* in I_h, may be capable of inhibiting the effects of activation of β-adrenergic or serotonergic receptors. This possibility was investigated *in vitro* in non-oscillating LGND neurones. Ba^{2+} (600–800 μM) was included in the bathing solution to block the adenosine-mediated increase in K^+ conductance and to isolate adenosine-induced effects on I_h. Under current-clamp conditions, the neurones were held near the normal resting potential (e.g. -64 mV in the cell of Fig. 8), and were stimulated by periodic (1–2 Hz) injection of a small hyperpolarizing current pulse (50–120 ms duration) to mimic rhythmically occurring IPSPs. Each membrane hyperpolarization de-inactivated the low-

threshold Ca^{2+} spike, which was elicited as a rebound at the break of the current pulse, resulting in periodic hyperpolarization–Ca^{2+} spike cycles (Fig. 8*A* and *B*1). Under these conditions, local application of the β-adrenergic agonist isoprenaline (100–250 μM) resulted in a small depolarization and a decrease in apparent input

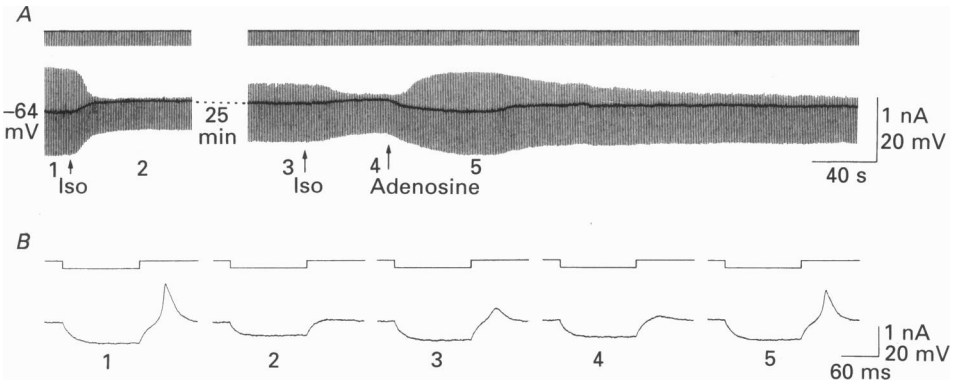

Fig. 8. Adenosine inhibits the β-adrenergic response of a LGND relay neurone. *A*, the neurone was held at −64 mV (as indicated), and hyperpolarizing constant current pulses (−0·25 nA, 120 ms, 1 Hz) were injected. Hyperpolarizing voltage deflections as a response to the current pulse indicate the apparent input resistance, and a Ca^{2+} spike is elicited as rebound following de-inactivation by the preceding hyperpolarization. Numbers depict examples that are shown at a faster time sweep for comparison in *B*; *B*1 represents control conditions. Local application of isoprenaline (Iso, 200 μM) elicits a small depolarization from −64 mV associated with a marked decrease in input resistance, due to an increase in the portion of I_h that is activated at −64 mV. As a secondary effect, the rebound Ca^{2+} spike is reduced, due to incomplete de-inactivation (*B*2). Only partial recovery from isoprenaline has occurred after 25 min (*B*3), and a further application of isoprenaline results in a small depolarization and decrease in resistance as before (*B*4). Local application of adenosine (3 mM) results in a small hyperpolarization and increase in apparent input resistance to a level similar to that obtained under control conditions. The hyperpolarizing response to the current pulse removes enough inactivation of the Ca^{2+} spike, and a fully grown Ca^{2+} rebound spike is again elicited (*B*5, compare with *B*1). Note that the effect of adenosine is more transient than that of isoprenaline. Bathing medium contained Ba^{2+} (600 μM) and TTX (1 μM). Upper traces are current, lower traces are membrane potential.

resistance (Fig. 8*A* and *B*1, 2), due to enhancement of the portion of I_h that is activated at resting potential (McCormick & Pape, 1990*b*). Due to the increased conductance, the hyperpolarizing response was decreased in amplitude, de-inactivation of the low-threshold Ca^{2+} spike was incomplete, and the rebound excitation was no longer elicited (Fig. 8*B*2, compare with *B*1). Even as long as 25 min after stimulation of the β-adrenergic receptors, the responses had only incompletely recovered (Fig. 8*A* and *B*3). A further application of isoprenaline resulted in a small depolarization, a decrease in apparent input resistance, and blockage of rebound excitation as before (Fig. 8*A* and *B*4). Local application of adenosine (2–5 mM, local) under these conditions readily and reversibly inhibited the β-receptor induced effects, in that due to the decrease in I_h the membrane slightly hyperpolarized and the apparent input resistance increased to control levels, allowing enough Ca^{2+} current de-inactivation to occur and hence a fully grown rebound Ca^{2+} spike to be

generated (Fig. 8*A* and *B*5, compare with *B*1). Similarly, application of adenosine inhibited the effects of serotonergic receptor stimulation (not shown).

These data indicate that activation of A_1 receptors may be capable of inhibiting β-adrenergic (or serotonergic) receptor-mediated dampening of rhythmic rebound burst firing in LGND neurones.

DISCUSSION

The results of the present study indicate that in LGND relay neurones adenosine induces two prominent effects: an increase of a membrane K^+ conductance, which is shared by A_1 and $GABA_B$ receptors, and a decrease of the hyperpolarization-activated cation current, I_h, probably mediated via inhibition of adenylyl cyclase activity.

Both of these effects appear to be mediated through activation of A_1 receptors, since they were reversibly blocked by a selective A_1 receptor antagonist (DPCPX), and imitated by agonists with high selectivity for the A_1 binding site (CPA, CHA). The increase in K^+ conductance elicited by NECA or CPCA, potent agonists for the A_2 binding site (Daly, 1982; Bruns *et al.* 1986), was also blocked by the selective A_1 antagonist DPCPX. Since the selectivity of these A_2 agonists is still a matter of controversy, and particularly NECA is assumed to selectively bind to A_2 receptors only during absence, blockage or occupation of A_1 receptors (as discussed in Williams, 1987), this result and the finding that the more selective A_2 receptor agonists DPMA (Bridges *et al.* 1988) and CGS 21680 (Hutchison *et al.* 1989) did not exert a consistent effect, indicate involvement of A_1 receptors. The A_1 receptors mediating the increase in K^+ conductance and the decrease in I_h are presumably postsynaptically located on the recorded neurones, since the responses persisted during blocked synaptic transmission in a TTX or low Ca^{2+}–high Mg^{2+}-containing solution. Furthermore, the increase in I_h evoked by stimulation of β-adrenergic receptors on LGND neurones through a near maximal application of a β-specific agonist was inhibited through application of adenosine, which is not consistent with a presynaptic reduction of release by adenosine of an endogenous transmitter substance. These conclusions are in agreement with results from autoradiographic studies, showing no effect of cerebral cortical ablation or enucleation on the dense pattern of [^{3}H]CHA grains in the LGN, and which were taken as evidence that adenosine A_1 receptors are not located on terminals of the corticothalamic or retinogeniculate pathways (Goodman *et al.* 1983).

Adenosine modulates two membrane conductances in LGND relay neurones

The most prominent response of LGND neurones to adenosine is a membrane hyperpolarization, which is due to an increase in membrane K^+ conductance, as is indicated by (1) the increased membrane conductance during the response, (2) the reversal potential of the adenosine-induced current, which is dependent on the K^+ gradient similar to that predicted by the Nernst equation for a K^+ electrode, (3) the blockage of the response by the K^+ channel blocker Ba^{2+}, and (4) the non-additivity of the adenosine-induced current with the K^+ current generated through activation of $GABA_B$ receptors. This result indicates convergence onto the same type of current,

and $GABA_B$ receptor activation generates a K^+ current in LGND neurones (Soltesz, Lightowler, Leresche & Crunelli, 1989).

Responses to adenosine associated with an increase in membrane K^+ conductance have been found in neurones of different brain regions, including the striatum, the neocortex, and the hippocampus (Trussell & Jackson, 1987; Gerber *et al.* 1989; McCormick & Williamson, 1989). In hippocampal CA1 pyramidal neurones the same K^+ conductance has been proposed to be shared by adenosine, $GABA_B$ and 5-HT_{1A} receptors via a pertussis-toxin-sensitive G-protein (Andrade *et al.* 1986; Nicoll, 1988; Zgombick *et al.* 1989). The K^+ channels which are activated through stimulation of A_1 receptors in LGND neurones seem to resemble those of CA1 pyramidal cells, in that they appear to be shared by $GABA_B$ and adenosine receptors, although a possible dependence on a GTP-binding protein remains to be delineated.

The blockage of K^+ conductances unmasks another response of LGND neurones to adenosine, which can be assumed to be due to a reduction of the hyperpolarization-activated cation current, I_h, based upon the following observations: (1) during adenosine the input conductance decreases in a voltage range negative to the normal resting potential, (2) the voltage dependence is very similar for the adenosine-induced response and I_h, (3) extracellular Cs^+, but not Ba^{2+}, blocks both the adenosine-activated current and I_h, (4) during action of adenosine the amplitude and rate of rise of I_h are substantially decreased, whereas instantaneous currents with no contribution of the hyperpolarization-activated conductance are not significantly influenced, and (5) addition of adenosine inhibits the increase in I_h mediated by stimulation of β-adrenoceptors.

In cardiac sino-atrial node myocytes, activation of β-adrenergic receptors leads to an increase in the hyperpolarization-activated cation current, termed I_f (DiFrancesco, Ferroni, Mazzanti & Tromba, 1986), while activation of muscarinic receptors decreases I_f, probably through an alteration in the sensitivity of the underlying conductance to membrane voltage (DiFrancesco & Tromba, 1988*a*). Though in the present study the voltage dependence of the conductance underlying I_h during stimulation of adenosine receptors could not be reliably determined, some indirect evidence suggests a negative shift along the voltage axis of the activation curve. Firstly, adenosine or CPA tended to maximally reduce I_h around the half-activation range, rather than equally depressing I_h over the whole voltage range tested. Secondly, the time constant of activation of I_h is highly voltage-dependent (McCormick & Pape, 1990*a*), and was found to be substantially increased during stimulation of A_1 receptors. Thirdly stimulation of A_1 adenosine and β-adrenergic receptors appears to be effective on I_h in an additive manner, in that application of CPA or adenosine inhibited the isoprenaline-induced increase in I_h (and vice versa). This finding suggests a mechanism of action of adenosine/CPA opposite to that of isoprenaline, and stimulation of β-adrenergic receptors results in a positive shift of the I_h activation curve on the voltage axis in LGND neurones (McCormick & Pape, 1990*b*).

Second messenger systems involved in responses to adenosine

The primary second messenger system associated with adenosine receptor activation appears to be the adenylyl cyclase–cyclic AMP system (Williams, 1987), with the two receptor sites being antagonistically coupled to adenylyl cyclase

activity: stimulation of A_1 receptors inhibits adenylyl cyclase activity resulting in a decrease of the intracellular level of cyclic AMP, whereas stimulation of A_2 sites increases the concentration of cyclic AMP due to stimulation of adenylyl cyclase activity (Van Calker *et al.* 1979). However, it has found to be difficult to determine the relationship between electrophysiological responses to adenosine, underlying cellular mechanisms and modulation of adenylyl cyclase activity (as discussed in Dunwiddie, 1985; Nicoll *et al.* 1990).

Three lines of evidence in the present study indicate that in LGND neurones the A_1 receptor-mediated inhibition of adenylyl cyclase activity leads to a decrease in I_h: (1) activation of A_1 receptors inhibits the increase in I_h after β-adrenoceptor stimulation, which has been shown to be mediated via an increased level of intracellular cyclic AMP, presumably due to adenylyl cyclase activation (McCormick & Pape, 1990*b*), (2) application of the adenylyl cyclase stimulant forskolin increases I_h and inhibits the A_1-induced decrease in I_h, and (3) application of the adenylyl cyclase inhibitor 2′,3′-dideoxyadenosine (Londos & Wolff, 1977; Johnson *et al.* 1989) imitates the reduction of I_h obtained during A_1 receptor stimulation. The finding that 2′,3′-dideoxyadenosine, A_1 agonists, or adenosine are effective on I_h regardless of any previous activation of adenylyl cyclase (e.g. through β-receptor stimulation) suggests that the basal activity of adenylyl cyclase may be high in the LGND slice preparation. Tonic stimulation of monoaminergic or other receptors that are positively coupled to adenylyl cyclase activity may contribute to the high basal activity. However, the latter hypothesis is not supported by the finding that application of β-adrenergic or serotonergic antagonists did not evoke a consistent effect on their own (H.-C. Pape, unpublished observations).

In conclusion, the results indicate that in LGND neurones, the hyperpolarization-activated cation current is regulated through the basal activity of adenylyl cyclase and hence the intracellular concentration of cyclic AMP. Adenosine A_1 and β-adrenergic (or serotonergic) receptors, by controlling the internal cyclic AMP level, may be capable of reciprocally modulating I_h, presumably through an alteration of its sensitivity to membrane voltage. A similar relationship has been described in sino-atrial node cells, where an opposite regulation of the cyclic AMP level by muscarine and catecholamines regulates the voltage dependence of I_f (DiFrancesco & Tromba, 1988*a*, *b*). This gating of I_f was found to occur through a mechanism independent of channel phosphorylation (DiFrancesco & Tortora, 1991). Though the exact processes underlying cyclic AMP-mediated regulation of I_h in LGND neurones need to be delineated, the finding that the protein kinase inhibitor H8 did not inhibit modulation of I_h suggests a mechanism, which may be independent of protein kinase activity.

Possible functional consequences of an increased level of adenosine in the LGND

Neuroanatomical tracing, stimulation and microionophoretic studies have shown that the ascending brainstem system is capable of gating the flow of sensory information through the thalamus depending on various states of sleep and arousal (as reviewed in McCormick, 1989; Steriade & McCarley, 1990). The major brainstem thalamic projection system comprises cholinergic fibres from the pendunculopontine and lateral dorsal tegmental nuclei, noradrenergic fibres from the locus coeruleus, and a serotonergic projection from the raphe nuclei. The activity of these brainstem

neurones has been found to vary in a state-dependent manner, in that the firing rates increase with an increased level of arousal or vigilance of the animal, presumably resulting in a varying release of the respective neurotransmitters in the thalamus.

Thalamocortical relay neurones are relatively hyperpolarized during periods of electroencephalographic synchronization (Hirsch *et al.* 1983). The low-threshold Ca^{2+} spike is de-inactivated (Jahnsen & Llinás, 1984*a*, *b*; Steriade & Deschênes, 1984), and the pattern of activity is dominated by Ca^{2+}-mediated burst activity, which can occur in a rhythmic or non-rhythmic manner as 1–2 Hz oscillation and spindle waves (Lamarre *et al.* 1971; McCarley *et al.* 1983; Steriade & Deschênes, 1984; Steriade & Llinás, 1988; Steriade *et al.* 1990*a*). During this mode of activity, the reliable relay of incoming synaptic information is degraded, presumably due to the presence of on-going burst discharges (McCormick & Feeser, 1990). Increased activity of the ascending brainstem system, associated with periods of a desynchronized electro-encephalogram, is capable of effectively dampening spindle oscillations and burst activity in the thalamus (Steriade & Llinás, 1988; McCormick, 1989; Steriade *et al.* 1990*a*). Thalamocortical neurones are relatively depolarized, the low-threshold Ca^{2+} spike is inactivated, and the value of the membrane potential is closer to threshold for generation of Na^+/K^+ action potentials (Hirsch *et al.* 1983; Steriade & Llinás, 1988; Steriade *et al.* 1990*a*). In the LGND, these states of increased brainstem activity are associated with a rise in overall activity, an increase in the ratio with which retinal signals are transferred to relay neurones, and an improved faithfulness of visual signal transmission in terms of improved contrast information (Steriade, Paré, Hu & Deschênes, 1990*b*).

The exact mechanisms for these changes in geniculocortical transmission during activity of the ascending brainstem system are unknown. However, the intrinsic electrophysiological properties of thalamocortical neurones appear to be important. For example, activation of α_1-adrenergic and also muscarinic receptors in geniculocortical relay neurones induces a decrease in membrane K^+ conductance (McCormick, 1989). The resulting depolarization tends to move the membrane potential out of a range of Ca^{2+}-mediated burst activity into a region closer to single spike firing. Activation of β-adrenergic or serotonergic receptors in these neurones results in a shift to more positive voltage of I_h activation (McCormick & Pape, 1990*b*). This modulation of I_h effectively interrupts intrinsic 1–2 Hz oscillation, which critically depends upon an interaction in the time and voltage domain between I_h and the low-voltage-activated Ca^{2+} current (McCormick & Pape, 1990*a*, *b*). Furthermore, this effect of monoaminergic receptor activation has been proposed to be particularly important for the dampening of spindle oscillations in the thalamus. Spindling consists of re-occurring cycles of Ca^{2+}-mediated burst activity and relies heavily upon recurrently mediated IPSPs in thalamocortical neurones, which provide the membrane hyperpolarization necessary for de-inactivation of the low-threshold Ca^{2+} current (Steriade & Deschênes, 1984). The enhancement of I_h at a given membrane potential during stimulation of β-adrenergic or serotonergic receptors increasingly limits duration and amplitude of the hyperpolarizing membrane responses, and thereby abolishes Ca^{2+}-mediated burst activity by preventing sufficient de-inactivation of the low-threshold Ca^{2+} current to occur (Pape & McCormick, 1989; McCormick & Pape, 1990*b*). In this manner, increased activity

of the ascending brainstem system associated with periods of wakefulness may interrupt Ca^{2+}-mediated burst activity in thalamocortical neurones and facilitate a mode of tonic repetitive activity of fast action potentials which allows more faithful transmission of incoming synaptic information (Steriade & Llinás, 1988; Steriade *et al.* 1990*a*).

The results of the present study indicate that in LGND neurones these responses to the activating brainstem system may not or to a lesser degree be evoked during increased levels of adenosine. Activation of A_1 receptors leads to a decrease in I_h, and thereby inhibits the increase in I_h which is associated with β-adrenergic (or serotonergic) receptor activation. This action of adenosine was found to potently block the dampening effect of β-adrenoceptor or serotonergic receptor activation on rebound burst activity. High levels of extracellular adenosine occurring in the thalamus *in vivo* may thus be capable of abolishing the dampening influence on oscillatory burst activity of the ascending monoaminergic brainstem system. Concomitantly with the decrease in I_h, activation of A_1 receptors results in a great increase in K^+ conductance. Under the experimental conditions of the present study, the membrane hyperpolarization resulting from the increase in K^+ conductance was found to be sufficiently large to move the neurones from a region close to threshold for Na^+/K^+-mediated single spike firing into a region where the low-threshold Ca^{2+} current is partly de-inactivated. Activation of A_1 receptors as such may be capable of directly promoting Ca^{2+}-mediated burst activity in thalamocortical neurones. In any case, the increase in membrane K^+ conductance associated with A_1 receptor activation can be assumed to functionally counteract the decrease in conductance for K^+ ions resulting from activation of α_1-adrenergic and/or muscarinic receptors. In addition, the largely increased input conductance of the cells during activation of A_1 receptors probably reduces the responsiveness of the membrane potential to current flow, resulting, for example, from synaptic inputs to LGND relay neurones of various origin, such as the retina, the visual cortex, intrathalamic sources, or the brainstem. The apparently little voltage sensitivity of the A_1 receptor-induced K^+ conductance, and the lack of desensitization of the response during repeated activation, suggest that the depressant action of adenosine may function over wide ranges in the voltage and time domain.

While activation of A_1 receptors thus appears to exert prominent postsynaptic effects, and enzymatic systems for metabolism and re-uptake of adenosine have been demonstrated to exist (Wu & Phillis, 1984), comparatively little is known about the source of adenosine that mediates these responses in the intact brain. Several factors may contribute to the regulation of adenosine level (reviewed in Dunwiddie, 1985). Adenosine has been found to be synaptically released in a stimulation-dependent way (Schubert *et al.* 1976), but the mediating pre- or postsynaptic elements have not yet been identified. One important parameter in determining the adenosine level appears to be the metabolic state of the cells. For example, increasing the electrical activity or the general metabolic rate of the brain, or decreasing the supply of metabolic precursors or oxygen, has been found to result in an increase in the extracellular concentration of adenosine of several orders of magnitude (Dunwiddie, 1985). Thus it appears as if non-uniformity between the demand for high energy metabolites during neuronal activity and the supply of energy through the metabolic

pool of the cells leads to an increased production of adenosine. Unfortunately, available data do not allow us to speculate about differences in energy consumption between different modes of neuronal activity, such as burst and single spike firing in the thalamus. However, the present results indicate that an increased level of extracellular adenosine depresses high frequency single spike firing and effectively dampens responsiveness of geniculocortical neurones to activating brainstem influences, thereby promoting a state of burst activity. If adenosine indeed serves as a regulating feedback mechanism between the metabolic demand of neuronal activity and the availability of energy sources, the present data may indicate that during periods of reduced energy supply a state of burst activity is established in geniculocortical neurones, which may contribute to an energy-conserving function.

Whether or not these alterations in thalamocortical firing mode are capable of modulating the onset or maintenance of sleep, cannot be determined on the basis of available results. However, behavioural studies indicate that the level of adenosine may be an important factor in determining the sleep–waking cycle. Systemic application of adenosine agonists to rats has been found to increase the duration of slow-wave and deep sleep, to increase or decrease paradoxical sleep depending upon the doses administered, and to decrease wakefulness (Radulovacki, Miletich & Green 1982; Radulovacki *et al.* 1984). The inhibition of metabolic degradation of adenosine could induce sleep-like states (Mendelson, Kuruvilla, Watlington, Goehl, Paul & Skolnick, 1983; Radulovacki, Virus, Djuricic-Nedelson & Green, 1983), and endogenous levels of adenosine in the brain rise substantially in sleep-deprived rats (Haulica, Ababei, Branisteanu & Topoliceanu, 1973). Since administration of adenosine agonists by itself was not found to induce sleep, it was concluded that adenosine subserves a modulatory role rather than a direct function in the sleep process (Dunwiddie, 1985).

We suggest that adenosine may have the ability to inhibit the responsiveness of thalamocortical relay neurones to activating brainstem influences, and thereby not only to regulate the state of activity in the thalamus but also to participate in the modulation of the sleep–waking cycle. This control by adenosine of thalamocortical activity may reflect an adaptive mechanism, which is governed by the metabolic state of the neurones.

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