A1 Adenosine Receptors Modulate Respiratory Activity of the Neonatal Mouse Via the cAMP-Mediated Signaling Pathway

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Mironov, S. L., K. Langohr, and D. W. Richter. A1 adenosine receptors modulate respiratory activity of the neonatal mouse via the cAMP-mediated signaling pathway. J. Neurophysiol. 81: 247–255, 1999. The effects of adenosine and its analogs on the function of the respiratory center were studied in the spontaneously active adult respiratory network of neonatal and juvenile mice (4–14 days old). Whole cell, spontaneous postsynaptic currents (sPSCs) and single channel KATP currents were recorded in inspiratory neurons of the pre-Bötzinger complex. Adenosine (50–600 μM) inhibited the respiratory rhythm. This was accompanied by an increase in the activity of KATP channels in cell-attached patches. The A1 adenosine receptor agonist, 2-chloro-N6-cyclopentyladenosine (CCPA, 0.3–2 μM), inhibited the respiratory rhythm, sPSCs, and enhanced activity of KATP channels. The A1 adenosine receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 1–3 μM), showed opposite effects and occluded the CCPA actions. Agents specific for A2a adenosine receptors (CGS 21860 and NECA, both applied at 1–10 μM) were without effect. Elevation of intracellular cAMP concentration ([cAMP]) by 8-Br-cAMP (200–500 μM), forskolin (0.5–2 μM), or isobutylmethylxanthine (IBMX, 30–90 μM) reinforced the rhythm, whereas NaF (100–800 μM) depressed it. The open probability of single KATP channels in cell-attached patches decreased after application of forskolin and increased in the presence of NaF. [cAMP], elevation reversed the effects of A1 receptors both on the respiratory rhythm and KATP channels. A1 receptors and [cAMP], modified the hypoxic respiratory response. In the presence of A1 agonists the duration of hypoxia augmented, and depression of the respiratory rhythm occurred earlier. Elevation of [cAMP], prolonged augmentation and delayed the development of the depression. We conclude that A1 adenosine receptors modulate the respiratory rhythm via inhibition of intracellular cAMP production and concomitant activation of KATP channels.

INTRODUCTION

Adenosine is an ubiquitous neuromodulator that acts through specific membrane receptors: A1, A2 (further subdivided into high-affinity A2a and low-affinity A2b types) and A3 receptors (Fredholm 1995; Olsson and Pearson 1990). All these receptors are coupled to G-proteins, which can inhibit or stimulate adenylyl cyclase. Resulting changes in intracellular cAMP levels can cause up- or down-regulation of various ion channels (Gerber and Gähwiler 1994; Kuroda et al. 1976). Modulation of ion channels can be also directly accomplished by β,γ subunits of G-proteins via membrane-delimited pathways (Dolphin et al. 1986; Olsson and Pearson 1990).

In the CNS, adenosine and its analogues depress neuronal firing rates (Dunnwiede 1985; Greene and Haas 1989; Meghji 1991; Olsson and Pearson 1990) by acting postsynaptically through an A1 receptor-controlled increase of voltage- or Ca2+-dependent K+ conductances (Gerber and Gähwiler 1994; Greene and Haas 1985). This involves cAMP- and G-protein–mediated signaling pathways (Thompson et al. 1993). Presynaptic terminals also have A1 receptors, which suppress transmitter release through inhibition of Ca2+ currents (Dolphin et al. 1986; Scholz and Miller 1991) and by potentiating K+ conductances (Trussell and Jackson 1987).

Adenosine receptors are differently expressed in the brain. High densities of A1 receptors are found in areas that are essential for respiratory control (Reppert et al. 1991). Several lines of evidence also point to a critical role of adenosine in the hypoxic depression of ventilation. Systemic administration of adenosine receptor agonists depresses respiration in vivo, which is antagonized by theophylline or aminophylline (Eldridge et al. 1985; Ginsborg and Hirst 1972). 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), an antagonist to A1 adenosine receptors, enhances the respiratory rhythm in cats even during normoxia, revealing tonic activation of A1 receptors (Schmidt et al. 1995). Activation of A1 adenosine receptors and hypoxia produces similar depression of synaptic interactions. Because extracellular levels of adenosine are increased during prolonged hypoxia (Lutz et al. 1992; Nagel et al. 1993), it was suggested that adenosine receptors contribute to the failure of synaptic interaction observed during hypoxia (Richter et al. 1991). A1 receptor antagonists, such as theophylline (Eldridge et al. 1985) and DPCPX (Schmidt et al. 1995), delay such hypoxic depression of respiratory activity.

The aim of the present study was to investigate the mechanisms of the adenosinergic modulation of respiratory neurons in vitro and their possible involvement in the hypoxic reactions of the respiratory network. The use of isolated respiratory center (Smith et al. 1991) allowed us to study the influence of adenosine on the function of the respiratory network that is not contaminated by indirect effects originating from failure of cardiovascular functions, and hence brain stem metabolic supplies, or from peripheral and central chemoreceptors. Based on the current knowledge about the action of adenosine in the CNS, the study was focused on spontaneous synaptic currents and K+ channels in inspiratory neurons. It was found that adenosinergic effects on the respiratory rhythm could be mimicked by activation of A1 receptors and were associated with activation of KATP channels. Manipulations of the intracellular cAMP concentration ([cAMP]) altered the respiratory rhythm and modulated the open probability of single KATP channels in cell-attached patches. Because the effects caused by activation of A1 receptors were neutralized by [cAMP], elevation, we suggest
that adenosine’s effects on the respiratory rhythm can be explained by changes in [cAMP], which alter the function of KATP channels.

METHODS

Slice preparations

Mice (NMRI) of both sexes (P4–P14) were anesthetized with ether and decapitated at the C3–C4 spinal level. The brain and upper cervical spinal cord were isolated in ice-cold artificial cerebrospinal fluid (ACSF) that was saturated with carbogen (95% O2–5% CO2). Following a transverse cut of the neuroaxis at the level of the inferior colliculus, the cerebellum was removed. The isolated brain stem was glued with cyanocrylate on an agar block with its rostral end directed upwards. Brain stem slicing was started from the rostral end with the neuroaxis inclined by 20° to the plane of the blade. Such configuration kept most transverse projections from pre-Bötzinger complex (preBoC) to the XII nucleus and their axons to XII rootlets intact (Ramírez et al. 1996). On sectioning the brain stem, the caudal end of the aqueduct was reached. Thereafter slices were cut 100–200 μm thick until cytoarchitectonic landmarks such as inferior olive, nucleus of the solitary tract, hypoglossal nucleus and nucleus ambiguous were visible while the facial nucleus disappeared, indicating that the rostral boundary of the preBoC was reached. The next cut was made 650 ± 750 μm thick to obtain one slice containing the functional respiratory center. This slice was transferred into the recording chamber, put onto the nylon mesh and fixed with a horseshoe-shaped holder, and the XII rootlet was drawn into a suction electrode. The concentration of extracellular K+ in ACSF saturated with carbogen at 29°C was elevated to 8–10 mM within 30–60 min to activate the KATP channels. This produced symmetric openings seen during a given measurement. These estimates of ATP-sensitivity of 75 pS-channels was veriﬁed in nine inside-out patches because there were also ‘‘sleepy’’ channels that could be activated by diazoxide (Fig. 5).

The properties of KATP channels in inspiratory neurons were described previously (Mironov et al. 1998). In the present study the cells were identiﬁed by their discharge patterns, showing correlation of spontaneous activity, even when O2 pressure had been reduced for 20–30 times for a given slice with a complete restoration of respiratory rhythmic activity, even when O2 pressure had been reduced for 20–30 min.

Electrophysiological recordings

Activity from XII rootlets was recorded with suction electrodes, ampliﬁed 5,000–10,000 times, band-pass (0.25–1.5 kHz) ﬁltered, rectiﬁed, and integrated (Paynter ﬁlter with a time constant of 50–100 ms). Hypoglossal activity was taken as index of the central respiratory rhythm (Smith et al. 1991). Intracellular recordings were obtained from preBoC inspiratory neurons using patch electrodes manufactured from borosilicate glass with ﬁlament (GC150F, Clark Instruments, UK). They had tip openings of 1.5–2 μm and resistances of 2–4 MΩ. Intracellular signals were ampliﬁed with a patch-clamp ampliﬁer EPC-7 (ESF Friedland, Germany). Membrane currents were ﬁltered at 3 kHz (−3 dB), digitized at 5 kHz, and stored for analysis with the use of an IBM-compatible PC. Data analysis was performed with the use of home-written programs in Turbo-Pascal 7.0. Data are presented as means ± SE. Statistical signiﬁcance was determined by using Student’s t-tests. Results were considered signiﬁcant if P < 0.05.

Respiratory neurons were searched in the area attributed to the preBoC (Smith et al. 1991). A ‘‘blind patching’’ technique (Blanton et al. 1989) was used, and inspiratory neurons were identiﬁed by their discharge patterns, showing correlation of spontaneous action potentials with inspiratory output of hypoglossal nerve. In about 80% of trials, gentle sucking led to formation of a gigaseal with a resistance of 3–12 GΩ [mean 6 ± 2 (SE) GΩ, n = 127]. Thereafter the spikes became larger and were seen as large deflections in the cell-attached mode (Fig. 1). After rupturing the patch, the whole cell conﬁguration was obtained, and the discharges were blocked revealing the underlying spontaneous excitatory synaptic currents (Fig. 3) (see also Mironov and Richter 1998; Mironov et al. 1998).

Only data obtained from inspiratory neurons are presented here. In comparison with other cells, e.g., expiratory and tonically active nonrespiratory neurons, the spikes recorded in cell-attached mode were absent in interburst intervals or after rhythmic activity was blocked. Therefore all single channel data were measured during the intervals between inspiratory bursts. The current and patch command potentials in cell-attached patches are presented as inside the cell minus outside, according to conventions for intracellular recordings. The open probability, Popen, was obtained by dividing the mean current by the unitary current and the number of channels. The latter was determined as a maximal number of simultaneous openings seen during a given measurement. These estimates of the number of active channels were also conﬁrmed by binominal analysis. They, however, may not correspond to the total number of channels present in the patch because there were also ‘‘sleepy’’ channels that could be activated by diazoxide (Fig. 5).

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Solutions and drugs

ACSF contained (in mM) 128 NaCl, 3 KCl, 1.5 CaCl₂, 1.0 MgSO₄, 21 NaHCO₃, 0.5 NaH₂PO₄, and 30 d-glucose (pH 7.4 was adjusted with NaOH). Solutions with elevated K⁺ (8–10 mM) were obtained by replacing NaCl with KCl. The pipette solution for cell-attached recordings contained (in mM) 125 KCl, 15 NaCl, 2 MgCl₂, 2 ATP, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 1 CaCl₂, 3 1,2-bis-(2-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid (BAPTA); pH was adjusted to 7.4 with KOH and osmolarity to 285–290 mOsm. For intracellular recordings, KCl was replaced by K⁺ gluconate. All salts used for extra- and intracellular solutions were from Sigma (Deisenhofen, Germany). Adenosine agonists and antagonists, forskolin, IBMX, and 8-Br-cAMP were obtained from Research Biochemical International (Cologne, Germany).

The bath solution was recycled into a 200 ml reservoir equilibrated with carbogen. All drugs were added from corresponding stock solutions, which were prepared freshly in ACSF or dimethyl sulfoxide (DMSO). The residual DMSO concentration was <0.1%, and the vehicle at 0.1% concentration had no effect. Depending on the perfusion rate (ranged from 25 to 45 ml/min), the drugs were delivered to the experimental chamber within 8–12 s. Complete washout of drugs was achieved by the perfusing of 400–500 ml fresh solution with the same K⁺ concentration.

RESULTS

A₁ receptors modulate the respiratory rhythm

One to two minutes after the application of adenosine (50–600 μM) to the bath, both the amplitude and the frequency of the hypoglossal rhythm decreased (Fig. 1). Under control conditions, the mean interval between XII inspiratory bursts was 5.0 ± 0.4 s (N = 26). In the presence of adenosine, it increased to 7.2 ± 0.4 s (50 μM, N = 7), 12.1 ± 0.6 s (200 μM, N = 8), and 18.2 ± 0.7 s (500 μM, N = 11), respectively. Here and below, N and n correspond to the number of slices and inspiratory neurons examined, respectively.

To identify the type of receptor responsible for this action, we applied the pharmacological agents, which act on specific adenosine receptor subtypes. Submicromolar concentrations of the A₁ receptor agonist, 2-chloro-N⁶-cyclopentyladenosine (CCPA), mimicked the action of adenosine on the respiratory rhythm (Fig. 2, first trace). In the presence of CCPA, the mean interval between hypoglossal inspiratory bursts was 7.0 ± 0.4 s (0.3 μM, N = 7), 11.2 ± 0.8 s (0.9 μM, N = 8), 16.1 ± 0.8 s (2 μM, N = 7), and 31.2 ± 1.7 s (4 μM, N = 9), respectively, compared with the control value of 5.1 ± 0.3 s (N = 21). In the presence of CCPA the amplitude of respiratory bursts decreased to 91 ± 2 (0.3 μM), 51 ± 6 (0.9 μM), 11 ± 3 (2 μM), and 3 ± 1% (4 μM) of control.

The respiratory bursts were augmented by A₁ receptors antagonist, DPCPX. This likely reveals a tonic inhibition of respiratory activity by endogenous adenosine. In the presence of 1 μM DPCPX, the interval between inspiratory bursts decreased from 6.5 ± 0.5 s to 3.5 ± 0.3 s, and their amplitude increased to 145 ± 8% of control (n = 11). In the presence of DPCPX, submicromolar concentrations of CCPA were no longer effective, and only at concentrations >10 μM the rhythm was suppressed again (n = 4, Fig. 2, third trace).

Neither of the agonists for A₂ receptors (Fredholm 1995), 2-p-(2-carboxyethyl)phenethylamino-5'-N'-ethylcarboxamidoadenosine (CGS 21860) (A₂₃₅), or 5'-N'-ethylcarboxamido-adenosine (NECA) (A₂₃₆), affected the rhythm, nor prevented

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**FIG. 1.** Response of K⁺ᵣᵣ channels and respiratory center to adenosine. Iₚ, top: recordings of the membrane current in a cell-attached patch XII, middle: integrated hypoglossal nerve activity. Patch command potential was 0 mV. Two arrows denoted as "c" and "o₁" indicate the closed and 1st open channel levels, respectively. Adenosine was applied at the beginning of the trace. Note correlation of spike discharges in the inspiratory neuron (sharp, vertical deflections) with the inspiratory bursts in hypoglossal nerve. Adenosine inhibited both spike discharges and the respiratory XII bursts, and in parallel, the channel activity increased. Bottom: representative traces of activity of single K⁺ᵣᵣ channels measured in the presence of adenosine at times indicated.

**FIG. 2.** Effects of A₁ receptor agonists (CCPA) and antagonists (DPCPX) on the hypoglossal nerve discharge. Drugs were applied at the beginning of the trace. Note that to suppress respiratory bursts in the presence of DPCPX, CCPA concentration had to be raised 60-fold.
Changes in spontaneous synaptic currents induced by activation of $A_1$ receptors

In whole cell mode inspiratory neurons revealed spontaneous inhibitory (sIPSC) and excitatory (sEPSC) postsynaptic currents. They had different voltage-dependency: sEPSCs (and synaptic drives, see below) reversed at $0 \pm 3$ mV and sIPSCs changed sign at $-59 \pm 5$ mV ($n = 23$). Thus at a holding potential of $-40$ mV, spontaneous IPSCs and EPSCs could be observed as brief upward and downward deflections from the baseline (Fig. 3). In inspiratory neurons, sEPSCs are assembled into synaptic drive currents (SDCs), which correlate with inspiratory bursts recorded from XII nerve (Fig. 3). sIPSCs were generated by both GABA$_A$- and glycine-receptors, as they were inhibited by $10 \mu$M bicuculline and $1 \mu$M strychnine. Either antagonist produced only a partial blockade, however, and only their combined application led to a complete inhibition of sIPSCs ($n = 13$, data not shown). CNQX ($4 \mu$M) abolished both sEPSCs and SDCs, indicating that they were generated by AMPA/kainate receptors ($n = 11$, data not shown).

As Fig. 3 shows, $1 \mu$M CCPA inhibited all types of synaptic currents, but with different efficacy, corresponding to the following sequence: SDCs > sEPSCs > sIPSCs. The changes in sIPSCs and sEPSCs induced by $1 \mu$M CCPA were analyzed at $-25$ and $-75$ mV, respectively, because at these potentials the contribution of oppositely directed synaptic currents was minimal. For five inspiratory neurons 5 min after CCPA addition, the mean amplitude and frequency of synaptic currents were $16 \pm 3$ pA and $6 \pm 3$ Hz versus $31 \pm 2$ pA and $25 \pm 2$ Hz in control (sIPSCs), $-12 \pm 2$ pA and $3 \pm 2$ Hz versus $-32 \pm 2$ pA and $24 \pm 3$ Hz (sEPSCs). In the presence of $1 \mu$M CCPA, the mean amplitude of synaptic drives recorded at $-50$ mV decreased to $-7 \pm 3$ pA from the control value of $-37 \pm 6$ pA.

$K_{ATP}$ channels are activated by $A_1$ receptors

Figure 1 shows that bath application of adenosine suppressed both spiking activity of neurons and inspiratory output of the hypoglossal nerve. This was accompanied by a gradual increase in the activity of single $K_{ATP}$ channels (for their identification see below and Mironov et al. 1998). Adenosine ($200 \mu$M) increased the open probability ($p_{open}$) from $0.06 \pm 0.02$ to $0.28 \pm 0.12$ ($n = 9$).

CCPA enhanced the activity of the channels, which was additionally increased by hypoxia (Fig. 4). CCPA ($1 \mu$M) increased $p_{open}$ from $0.13 \pm 0.07$ to $0.28 \pm 0.09$ and hypoxia increased it further to $0.42 \pm 0.12$ ($n = 11$). Addition of adenosine to the bath was no longer effective after CCPA was applied ($n = 4$, data not shown), which was likely because of occlusion of $A_1$ receptors.

As Fig. 4 shows, in the presence of CCPA and during hypoxia the channel conductance ($75$ pS for this patch) did not change more than in control (Fig. 4A, B). During hypoxia, however, the time distributions of synaptic events were quite different (Fig. 4C). The open time distributions were approximated by single exponentials. For five inspiratory neurons their mean values were $1.28 \pm 0.06$ ms (control), $1.29 \pm 0.07$ ms (1 $\mu$M CCPA), and $1.31 \pm 0.06$ ms (hypoxia).
FIG. 5. K<sub>ATP</sub> channels are potentiated by adenosine. Recordings were made after addition of 20 µM diazoxide (A), 200 µM adenosine (B), and 90 µM glibenclamide (C) to the bath. Horizontal dotted lines indicate the closed (top) and dashed lines indicate several open channel levels. The time after the drug addition is indicated near each trace (0 min corresponds to the control). The patch was held at 0 mV.

not change. Its mean value was 75 ± 5 pS (n = 34). Therefore changes in channel activity must involve modification of the gating mechanism. Because of the presence of several channels in cell-attached patches, only the open times could be quantified. Their distribution was monoeponential (Mironov et al. 1998). CCPA and hypoxia did not change the mean open time (Fig. 4), therefore the observed effects in P<sub>open</sub> stem from changes in the closed time distribution.

Bath application of specific activator of K<sub>ATP</sub> channels, diazoxide, increased the channel activity and recruited additional (sleepy) channels (Fig. 5A). Subsequent addition of 200 µM adenosine to the bath further enhanced the activity of K<sub>ATP</sub> channels (Fig. 5B). Diazoxide (20 µM) increased P<sub>open</sub> from 0.11 ± 0.06 to 0.34 ± 0.09 and adenosine (200 µM) raised it further to 0.44 ± 0.12 (n = 7). In the experiment shown in Fig. 5C, 90 µM glibenclamide, a blocker of K<sub>ATP</sub> channels, inhibited the channel activity. Similar effects were observed for four other cells. For cells pretreated with diazoxide, the activity of K<sub>ATP</sub> channels could be further enhanced by 0.5–3 µM CCPA (n = 8, data not shown). In the presence of 1 µM CCPA, P<sub>open</sub> increased from 0.23 ± 0.05 to 0.38 ± 0.09 (n = 4).

Adenosine and intracellular cAMP

As mentioned in the INTRODUCTION, adenosine actions can be mediated by intracellular cAMP. It was found that all modulations of intracellular cAMP concentration altered the respiratory rhythm (Fig. 6A). [cAMP], increase induced by forskolin (0.5–2 µM), an activator of adenylyl cyclase, enhanced the amplitude of inspiratory XII bursts, their frequency, and duration. Similar effects were observed after application of IBMX (30–90 µM), an inhibitor of cAMP-phosphodiesterase, and with application of the membrane-permeable analog, 8-Br-cAMP (200–500 µM). NaF (100–800 µM), which activates G<sub>i</sub>-proteins at submillimolar concentrations (Blackmore et al. 1985), decreased the frequency and amplitude of inspiratory bursts. Under control conditions, the mean interval between inspiratory bursts was 5.0 ± 0.4 s, and in the presence of cAMP-elevating agents, it decreased to 2.2 ± 0.2 s after forskolin (N = 26), to 2.1 ± 0.2 s after IBMX (N = 17), to 2.4 ± 0.3 s after 8-Br-cAMP (N = 7), or increased to 8.8 ± 0.4 s after NaF (N = 14). In the presence of forskolin, synaptic drives and sPSCs were potentiated (Fig. 6B).

Next, we examined whether K<sub>ATP</sub> channels could be ma-
FIG. 7. Sequential inhibition of K$_{ATP}$ channels by forskolin (1 µM) and glibenclamide (70 µM). Recordings were made in a cell-attached patch at a potential of 0 mV. Horizontal dotted lines indicate the closed (top) and dashed lines indicate open channel levels. Time (min) after the drug addition is indicated near each trace (0 min corresponds to the control). Glibenclamide was added in the presence of forskolin after the forskolin response had reached a steady-state.

Manipulated by changing [cAMP]$_i$ levels. Forskolin decreased the channel activity (Fig. 7). In the presence of 1 µM forskolin, $p_{open}$ decreased from 0.29 ± 0.11 to 0.09 ± 0.05 (n = 11). The channels were further inhibited and finally blocked by glibenclamide (n = 5; Fig. 7). NaF activated K$_{ATP}$ channels (Fig. 8). Five minutes after NaF was applied to the bath, action potential discharges disappeared and the activity of K$_{ATP}$ channels increased. These effects of NaF developed slowly and did not show an apparent dependence on drug concentrations that varied from 100 to 800 µM. Neither channel conductance nor its voltage-dependence was affected. NaF increased $p_{open}$ from 0.07 ± 0.04 to 0.31 ± 0.11 (n = 11).

cAMP elevation reversed the effects caused by activation of A$_1$ receptors. After addition of 1 µM CCPA, the frequency of hypoglossal nerve discharge decreased (Fig. 9A) and the activity of K$_{ATP}$ channels increased (Fig. 9B), but application of permeable cAMP analogue, 8-Br-cAMP (250 µM), reversed these effects (N = 4). Figure 9B also illustrates the inhibition of K$_{ATP}$ channels by ATP in an inside-out patch excised in the end of experiment.

In this study only the data obtained for inspiratory neurons are presented, but similar effects were observed in expiratory neurons (n = 18). For these cells the analysis was difficult to perform because on-going discharge activity considerably masked the channel activity and distorted the current baseline.

Adenosine and hypoxic response

The response of the respiratory center to hypoxia was biphasic. It consisted of augmentation that started ~1 min after the beginning of the hypoxic episode. An increase in frequency and amplitude of inspiratory bursts was transient and was followed by depression when the rhythm disappeared (Fig. 10). Enhancement of the rate of inspiratory bursts during early hypoxia was superimposed on a slowly developing DC-signal in integrated XII nerve discharge (Fig. 10) caused by enhanced tonic activity of hypoglossal moto-
The effects of forskolin on the respiratory response to hypoxia (Fig. 10B) were opposite to those observed after activation of A₁ receptors. In the presence of forskolin, the augmentation started earlier and lasted longer, resulting in delayed respiratory depression. CCPA restored the initial form of the hypoxic respiratory response. It should be noted that, at submicromolar concentrations (see Fig. 2), CCPA was no longer effective, and to change both the respiratory rhythm and its response to hypoxia in the presence of forskolin, the dose of CCPA had to be increased by about 70-fold (Fig. 10B, third trace; N = 7). Similar results were obtained with 10–50 μM IBMX (N = 12, data not shown).

In the presence of drugs, specific to K<sub>ATP</sub> channels, both the XII nerve activity and its hypoxic reaction were modified. Diazoxide (20 μM) increased the interval between inspiratory bursts from 5.4 ± 0.3 s to 8.0 ± 0.4 s, and the duration of augmentation shortened from 112 ± 18 s to 71 ± 12 s (N = 4). In the presence of 70 μM glibenclamide, the interval between inspiratory bursts decreased from 5.3 ± 0.4 s to 4.0 ± 0.4 s, and the duration of augmentation increased from 102 ± 18 s to 174 ± 32 s (N = 5). The inability to glibenclamide to abolish respiratory depression could be explained by loss of sensitivity of K<sub>ATP</sub> channels to glibenclamide during hypoxia (Mukai et al. 1998; Venkatesh et al. 1991) and/or contribution of other factor(s) (Hochachka et al. 1996) to respiratory depression.

**DISCUSSION**

Adenosine is a purinergic metabolite and a potent neuromodulator. Its various actions within the CNS were widely reviewed (Dunwiddie 1985; Fredholm 1995; Greene and Haas 1989; Meghji 1991; Olsson and Pearson 1990; von Lubitz et al. 1995). Adenosine receptors are subdivided into four types, which differ by their affinity to adenosine, pharmacology, and coupling to intracellular signaling pathways (Fredholm 1995; Olsson and Pearson 1990). All of them activate G-proteins, one target of which is the adenylyl cyclase, which is suppressed by A₁ receptors but is stimulated by A₂A and A₃ receptors. Resulting changes in [cAMP], can modify the function of various ion channels and receptors (Anholt 1994; Levitan 1988).

**Role of A₁ receptors and cAMP in rhythmogenesis**

It was shown (Schmidt et al. 1995) that A₁ receptors are functional in ventral respiratory neurons of the in vivo cat. The present study extends these findings in demonstrating that in neonatal mice A₁ receptors modulate the respiratory rhythm and its reaction to hypoxia. Elevation of [cAMP], had opposite effects, and the respiratory activity was changed by agents that alter intracellular cAMP levels either directly (8-Br-cAMP) or indirectly (forskolin, IBMX, and NaF). The effects of all substances tested in the present study are consistent with their presumed action on cAMP-signaling pathway. Similar effects of [cAMP], on respiratory rhythm generation were observed in brain stem–spinal cord preparation from newborn rats (Arata et al. 1993). These findings also corroborate the results obtained in the cat in vivo by Lalley et al. (1997), who demonstrated modulation of discharge patterns of respiratory neurons by protein kinase A.

**A₁ receptors and [cAMP], modulate K<sub>ATP</sub> channels**

A₁ receptors activated K<sub>ATP</sub> channels and the elevation of intracellular cAMP diminished their activity. The properties
of K\text{ATP} channels in inspiratory neurons were described previously (Mironov et al. 1998). These neurons have conductance of 75 pS, \( \rho_{\text{open}} \approx 0.1 \)–0.2 and \( \tau_{\text{off}} \approx 1\)–2 ms; therefore they can be attributed to a class of large conductance K\text{ATP} channels (Quayle et al. 1997). In cell-attached patches the channels were activated by hypoxia (Fig. 4) and diazoxide (Fig. 5) and were inhibited by glibenclamide (Figs 5 and 7). In inside-out patches the channels were inhibited by ATP (Fig. 9B). Interestingly in the cell line INS-1, K\text{ATP} channels (conductance of 50–70 pS) were blocked by tolbutamide and inhibited by forskolin, which led to enhanced insulin secretion (Ullrich et al. 1996).

The changes in the respiratory rhythm and the activity of K\text{ATP} channels induced by \( A_1 \) receptors and [cAMP], had similar kinetics and dependence on agonist concentration. Potentiation of K\text{ATP} currents by adenosine likely involves a G\text{R}-protein–mediated suppression of intracellular cAMP production. Recently it was shown that inspiratory motor discharges are suppressed by activation of G-protein–coupled receptors (Johnson et al. 1996). We suggest that a pathway that includes the activation of \( A_1 \) receptors, concomitant [cAMP], decrease leading to activation of K\text{ATP} channels, has particular physiological significance in inspiratory neurons, whereby respiratory rhythm and its hypoxic response is modified.

**Role of \( A_1 \) receptors and cAMP in hypoxia**

Extracellular adenosine originates from two sources (Meghji 1991). ATP, co-released from presynaptic terminals together with other neurotransmitters, is metabolized to adenosine by ectonucleotidases. Potentiation of the rhythmic activity by DPCPX (Fig. 2) indicates that the basal levels of adenosine are high enough to produce a tonic activation of \( A_1 \) receptors in neurons that participate in the control of respiratory rhythm. Another source of adenosine predominates during hypoxic conditions or intense neuronal activity and involves the production of adenosine from the metabolic breakdown of ATP. It rapidly crosses the membrane via a bidirectional transporter and acts extracellularly. K\text{ATP} channels are necessary for respiratory depression (Mironov et al. 1998). In inspiratory neurons, K\text{ATP} channels were activated by \( A_1 \) receptors, but hypoxia was able to further potentiate them. Although \( A_1 \) receptors contribute to the development of respiratory depression during hypoxia, their activation is not sufficient. For example, in the presence of DPCPX the depression was only delayed but not prevented.

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