

Summary of Research on Theophylline Treatment of Respiratory Deficiency

Wise Young, Ph.D., M.D.

W. M. Keck Center for Collaborative Neuroscience Rutgers University, Piscataway, New Jersey 08854 Web: http://sciwire.com, email: wisey@pipeline.com

Last updated May 7, 2003

Theophylline has long been used a a bronchodilator in the management of asthma (Aucoin, 1999). A member of a family of drugs called methylxanthines, theophylline relaxes smooth muscles, stimulates the central nervous system and cardiac muscles, and promotes diuresis. Theophylline and other members of the methylxanthine family, such as caffeine and aminophylline, are components of tea and coffee. Theophylline, however, is a methyxanthine that is also an adenosine receptor antagonist.

Methylxanthines inhibit phosphodiesterase, the enzyme that degrades cAMP (Ang & Antoni, 2002), and thus increases intracellular cAMP levels. Elevated levels of cAMP have been recently reported by Filbin, et al. (Cai, et al., 2002; Cai, et al., 2001; Cai, et al., 1999; Qiu, et al., 2002a; Qiu, et al., 2000; 2002b) to allow growing axons to overcome growth inhibitors in the central nervous system, such as Nogo and Mag. Also, cAMP directly increases excitability of presynaptic cation channels (Beaumont & Zucker, 2000). Forskolin, a stimulator of adenylate cyclase (the enzyme that makes cAMP), has marked effects on synaptic plasticity (Beck, et al., 2000; Storozhuk & Balaban, 1989) and adenyate cyclase is essential for long-term facilitation of synaptic function (Dixon & Atwood, 1989).

Nantwi, et al. (Nantwi, et al., 1996) reported that theophylline remarkably improved recovery of the respiratory diaphragm after hemisection of the C2 spinal cord. This injury essentially denervated the phrenic nucleus on one side. The phrenic nucleus is located at C3 and drives movements of the respiratory diaphragm. They found that a single injection of theophylline activated latent respiratory motor pathways to restore respiratory drive to phrenic motoneurons that had been denervated by the hemisection. In a subsequent study, Nantwi & Goshgarian (Nantwi & Goshgarian, 1998a) gave the drug for 3-30 days after injury and showed the 29 of 32 rats had maintained diaphragmatic activity at 30 days. This effect of theophylline appear to require theophylline's activity as an adenosine receptor antagonist because another methylxanthine enprofylline which

has no adenosine receptor antagonist activity did not improve respiratory function (Nantwi & Goshgarian, 1998b). Subsequent studies showed that other adenosine receptor antagonists have similar effects as theophylline (Basura, et al., 2002; Nantwi & Goshgarian, 2001; 2002).

Many other investigators have shown that adenosine A1 receptors modulate respiratory drive. Dong & Feldman (Dong & Feldman, 1995) had earlier shown that presynaptic adenosine A1 receptors depress inspiratory drive to phrenic motoneurons and that adenosine A1 receptor blockers increase inspiratory drive. Kawai, et al. (Kawai, et al., 1995) found that theophylline and 8-p-sulfophenyltheophylline both prevent hypoxic respiratory depression. These studies strongly confirm earlier work showing that adenosine inhibited respiration and that adenosine receptor antagonists prevented this effect (Eldridge, et al., 1985; Eldridge, et al., 1983; Krottmayer, et al., 1985; Millhorn, et al., 1984). Adenosine receptor antagonists, however, may contribute to seizure-induced damage in rats (Huang, et al., 2002) by increasing the excitability of neurons (Thummler & Dunwiddie, 2000). Adenosine has been shown to the mechanism by which increasing temperature suppresses neuronal excitability (Fujii, et al., 2000; Masino & Dunwiddie, 2000).

In summary, theophylline appears to have at least two beneficial effects on respiratory deficiency in conditions such as spinal cord injury. First, by blocking phosphodiesterase, it increases cAMP which allows axons to grow in the presence of growth inhibitors such as Nogo and MAG. Increased cAMP has also been implicated as a major mechanism of synaptic plasticity. Second, by blocking adenosine receptors which depresse presynaptic excitability, theophylline increases respiratory drive of phrenic neurons that have been partially denervated by spinal cord injury. The combined mechanisms of increasing presynpatic drive and facilitating growth and plasticity make theophylline a particularly attractive therapy for spinal cord injury. The animal data supporting its beneficial effects on respiratory recovery are strong and have been replicated in many laboratory. Theophylline is also drug with a long and proven track record of safety. Thus, it is a very attractive drug to test in clinical trial.

Literature Cited

• Ang KL and Antoni FA (2002). Functional plasticity of cyclic AMP hydrolysis in rat adenohypophysial corticotroph cells. Cell Signal 14:445-52. Summary: Characterisation of cyclic nucleotide-hydrolysing phosphodiesterases (PDEs) in recombinant systems has highlighted regulatory properties indicative of distinct physiological roles for these enzymes. The present study investigated the role of PDEs in the adenosine 3'5'-monophosphate (cAMP) response to the hypothalamic neuropeptides corticotrophin-releasing factor (CRF) and arginine vasopressin (AVP) in acutely dispersed rat adenohypophysial cells. Ca(2+)-activated PDE (PDE1) and Ca(2+)-independent, rolipram-sensitive PDE (PDE4) accounted for close to 90% of cAMP-hydrolysing activity in the adenohypophysis. Messenger RNA transcripts of PDE1 (isotypes 1A and 1C) and PDE4 (isotypes B and D3) were detected by RT-PCR. The PDE blockers rolipram and IBMX enhanced cAMP accumulation induced by CRF or CRF and AVP.

Vinpocetine, an inhibitor of low K(m) PDE1 isotypes, did not alter the response to CRF but enhanced the effect of the combined CRF/AVP stimulus. Thus, PDE4s terminate the cAMP response to moderate stimulation, while low-affinity PDE1 becomes important when the concentrations of CRF and AVP are characteristic of exposure to intensive stress. Department of Neuroscience, University of Edinburgh, 1 George Square, EH8 9JZ, Edinburgh, UK.

- Aucoin RG (1999). Respiratory pharmacology. http://pedsccm.wustl.edu/All-Net/english/pharmpage/resp/methylxan.html.
- Basura GJ, Nantwi KD and Goshgarian HG (2002). Theophylline-induced respiratory recovery following cervical spinal cord hemisection is augmented by serotonin 2 receptor stimulation. Brain Res 956:1-13. Summary: Cervical spinal cord hemisection leads to a disruption of bulbospinal innervation of phrenic motoneurons resulting in paralysis of the ipsilateral hemidiaphragm. We have previously demonstrated separate therapeutic roles for the ophylline, and more recently serotonin (5-HT) as modulators to phrenic nerve motor recovery; mechanisms that likely occur via adenosine A1 and 5-HT2 receptors, respectively. The present study was designed to specifically determine if concurrent stimulation of 5-HT2 receptors may enhance motor recovery induced by theophylline alone. Adult female rats (250-350 g; n=7 per group) received a left cervical (C2) hemisection that resulted in paralysis of the ipsilateral hemidiaphragm. Twenty-four hours later rats were given systemic theophylline (15 mg/kg, i.v.), resulting in burst recovery in the ipsilateral phrenic nerve. Theophylline-induced recovery was enhanced with the 5-HT2A/2C receptor agonist, (+/-)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI; 1.0 mg/kg). DOI-evoked augmentation of theophylline-induced recovery was attenuated following subsequent injection of the 5-HT2 receptor antagonist, ketanserin (2.0 mg/kg). In a separate group, rats were pretreated with ketanserin, which did not prevent subsequent theophylline-induced respiratory recovery. However, pretreatment with ketanserin did prevent DOI-induced augmentation of the theophyllineevoked phrenic nerve burst recovery. Lastly, using immunocytochemistry and in situ hybridization, we showed for the first time a positive co-localization of adenosine A1 receptor mRNA and immunoreactivity with phrenic motoneurons of the cervical ventral horns. Taken together, the results of the present study suggest that theophylline may induce motor recovery likely at adenosine A1 receptors located at the level of the spinal cord, and the concurrent stimulation of converging 5-HT2 receptors may augment the response. Department of Anatomy and Cell Biology, Wayne State University School of Medicine, 540 East Canfield Avenue, Detroit, MI 48201, USA.
- Beaumont V and Zucker RS (2000). Enhancement of synaptic transmission by cyclic AMP modulation of presynaptic Ih channels. Nat Neurosci 3:133-41. Summary: Presynaptic activation of adenylyl cyclase and subsequent generation of cAMP represent an important mechanism in the modulation of synaptic transmission. In many cases, short- to medium-term modulation of synaptic strength by cAMP is due to activation of protein kinase A and subsequent covalent modification of presynaptic ion channels or synaptic proteins. Here we show that presynaptic cAMP generation via serotonin receptor activation directly modulated hyperpolarization-activated cation channels (Ih channels) in axons. This modulation of Ih produced an increase in synaptic strength that could not be explained solely by depolarization of the presynaptic membrane. These studies identify a mechanism by which cAMP and Ih regulate synaptic plasticity. Division of

Neurobiology, Department of Molecular and Cell Biology, University of California, Berkeley, California 94720, USA. vahri@socrates.berkeley.edu

- Beck H, Goussakov IV, Lie A, Helmstaedter C and Elger CE (2000). Synaptic plasticity in the human dentate gyrus. J Neurosci 20:7080-6. Summary: Activitydependent plasticity is a fundamental feature of most CNS synapses and is thought to be a synaptic correlate of memory in rodents. In humans, NMDA receptors have been linked to verbal memory processes, but it is unclear whether NMDA receptor-dependent synaptic plasticity can be recruited for information storage in the human CNS. Here we have for the first time analyzed different forms of synaptic plasticity in human hippocampus. In human subjects who show a morphologically intact hippocampus that is not the primary seizure focus, NMDA receptor-dependent long-term potentiation (LTP) and forskolin-induced long-lasting potentiation are readily induced at the perforant pathdentate gyrus synapse. In this group, long-term potentiation could be partially depotentiated by low-frequency stimulation. Because patients with a hippocampal seizure focus showed a marked reduction in verbal memory performance in previous studies, we asked whether synaptic plasticity is similarly affected by the presence of a hippocampal primary seizure focus. We found that the amount of potentiation induced by highfrequency stimulation or perfusion of forskolin is dramatically reduced in this patient group. In addition, low-frequency stimulation is not effective in inducing synaptic depression. In summary, we show that activity-dependent synaptic plasticity with properties similar to the rodent is available for information storage in the human hippocampus. Because both verbal memory processes and synaptic plasticity are impaired by a hippocampal seizure focus, we suggest that impaired synaptic plasticity may contribute to deficient declarative memory in human temporal lobe epilepsy. Department of Epileptology, University of Bonn Medical Center, D-53105 Bonn, Germany. heinz@mailer.meb.uni-bonn.de
- Cai D, Deng K, Mellado W, Lee J, Ratan RR and Filbin MT (2002). Arginase I and polyamines act downstream from cyclic AMP in overcoming inhibition of axonal growth MAG and myelin in vitro. Neuron 35:711-9. Summary: Elevation of cAMP can overcome myelin inhibitors to encourage regeneration of the CNS. We show that a consequence of elevated cAMP is the synthesis of polyamines, resulting from an upregulation of Arginase I, a key enzyme in their synthesis. Inhibiting polyamine synthesis blocks the cAMP effect on regeneration. Either over-expression of Arginase I or exogenous polyamines can overcome inhibition by MAG and by myelin in general. While MAG/myelin support the growth of young DRG neurons, they become inhibitory as DRGs mature. Endogenous Arginase I levels are high in young DRGs but drop spontaneously at an age that coincides with the switch from promotion to inhibition by MAG/myelin. Over-expressing Arginase I in maturing DRGs blocks that switch. Arginase I and polyamines are more specific targets than cAMP for intervention to encourage regeneration after CNS injury. Biology Department, Hunter College, City University of New York, 695 Park Avenue, New York, NY 10024, USA.
- Cai D, Qiu J, Cao Z, McAtee M, Bregman BS and Filbin MT (2001). Neuronal cyclic AMP controls the developmental loss in ability of axons to regenerate. J Neurosci 21:4731-9. Summary: Unlike neonatal axons, mammalian adult axons do not regenerate after injury. Likewise, myelin, a major factor in preventing regeneration in the adult, inhibits regeneration from older but not younger neurons. Identification of the molecular

events responsible for this developmental loss of regenerative capacity is believed key to devising strategies to encourage regeneration in adults after injury. Here, we report that the endogenous levels of the cyclic nucleotide, cAMP, are dramatically higher in young neurons in which axonal growth is promoted both by myelin in general and by a specific myelin component, myelin-associated glycoprotein (MAG), than in the same types of neurons that, when older, are inhibited by myelin-MAG. Inhibiting a downstream effector of cAMP [protein kinase A (PKA)] prevents myelin-MAG promotion from young neurons, and elevating cAMP blocks myelin-MAG inhibition of neurite outgrowth in older neurons. Importantly, developmental plasticity of spinal tract axons in neonatal rat pups in vivo is dramatically reduced by inhibition of PKA. Thus, the switch from promotion to inhibition by myelin-MAG, which marks the developmental loss of regenerative capacity, is mediated by a developmentally regulated decrease in endogenous neuronal cAMP levels. Department of Biological Sciences, Hunter College, City University of New York, New York, New York 10021, USA.

- Cai D, Shen Y, De Bellard M, Tang S and Filbin MT (1999). Prior exposure to neurotrophins blocks inhibition of axonal regeneration by MAG and myelin via a cAMPdependent mechanism. Neuron 22:89-101. Summary: MAG is a potent inhibitor of axonal regeneration. Here, inhibition by MAG, and myelin in general, is blocked if neurons are exposed to neurotrophins before encountering the inhibitor; priming cerebellar neurons with BDNF or GDNF, but not NGF, or priming DRG neurons with any of these neurotrophins blocks inhibition by MAG/myelin. Dibutyryl cAMP also overcomes inhibition by MAG/myelin, and cAMP is elevated by neurotrophins. A PKA inhibitor present during priming abrogates the block of inhibition. Finally, if neurons are exposed to MAG/myelin and neurotrophins simultaneously, but with the Gi protein inhibitor, inhibition is blocked. We suggest that priming neurons with particular neurotrophins elevates cAMP and activates PKA, which blocks subsequent inhibition of regeneration and that priming is required because MAG/myelin activates a Gi protein, which blocks increases in cAMP. This is important for encouraging axons to regrow in vivo. Biology Department, Hunter College of the City University of New York, New York 10021, USA.
- Dixon D and Atwood HL (1989). Adenylate cyclase system is essential for long-term facilitation at the crayfish neuromuscular junction. J Neurosci 9:4246-52. Summary: Long-term facilitation (LTF), a form of synaptic plasticity demonstrated at the crayfish neuromuscular junction, is induced by tetanic stimulation and persists for hours. LTF can be divided into 2 phases: a tetanic phase, which occurs during stimulation, and a longlasting phase, which persists after stimulation. Activators and potentiators of cAMP (forskolin and 3-isobutyl-methyl-xanthine) produce facilitation of excitatory postsynaptic potentials, which attain approximately the amplitude of the long-lasting phase of LTF but last for a shorter time. Localized presynaptic injection of a protein inhibitor ("Walsh inhibitor") specific for the cAMP-dependent protein kinase blocks the long-lasting phase of LTF at synapses near the injection site with no apparent effect on the tetanic phase. Normal LTF develops and persists at synapses of the same axon distant from the injection site. Localization of the injected inhibitor was confirmed by fluorescent tagging. Localized injection of SQ22,536, an adenylate cyclase inhibitor, also blocks the second phase of LTF near the injection site, but not at distant synapses. These experiments establish a role for adenylate cyclase activation in the long-lasting phase of LTF. The

phosphatidylinositol second-messenger system is not important in LTF as inhibition of phospholipase C by injection of RA233, which blocks facilitatory effects of serotonin, does not affect any aspect of LTF. Department of Physiology, University of Toronto, Ontario, Canada.

- Dong XW and Feldman JL (1995). Modulation of inspiratory drive to phrenic motoneurons by presynaptic adenosine A1 receptors. J Neurosci 15:3458-67. Summary: The involvement and mechanisms of adenosine A1 receptors in regulating bulbospinal synaptic transmission of inspiratory drive to phrenic motoneurons were investigated. The adenosine analog N6-cyclopentyladenosine (CPA) induced a dose-dependent decrease of both inspiratory-modulated activity of C4 ventral roots and synaptic currents of phrenic motoneurons in an in vitro brainstem/spinal cord preparation from neonatal rats. No significant changes were observed in steady-state membrane current (during the expiratory phase). The depressant action of CPA on inspiratory drive was blocked by the selective A1 receptor antagonist 8-cyclopentyltheophylline (CPT). The adenosine receptor antagonist 3-isobutyl-1-methylxanthine (IBMX) induced varying degrees of enhancement of inspiratory-modulated synaptic current, as did CPT. This suggests a role of endogenous adenosine in synaptic transmission of respiratory drive to phrenic motoneurons. The relative contribution of pre- and postsynaptic adenosine receptors was examined by looking at the effects of CPA on postsynaptic membrane properties and on spontaneous or miniature excitatory postsynaptic currents (EPSCs). CPA had no detectable effect on the input resistance of phrenic moto-neurons. Moreover, the inward currents of phrenic moto-neurons in response to exogenously applied glutamate were not affected by adenosine-related compounds. On the other hand, CPA produced a significant decrease in the frequency of spontaneous and of miniature EPSCs. We conclude that adenosine can modulate transmission of inspiratory drive from bulbospinal neurons to phrenic motoneurons via presynaptic A1 receptors. Department of Physiological Science, University of California Los Angeles 90095-1527, USA.
- Eldridge FL, Millhorn DE and Kiley JP (1985). Antagonism by the ophylline of respiratory inhibition induced by adenosine. J Appl Physiol **59**:1428-33. Summary: The effects on respiration of an analogue of adenosine, L-2-N6-(phenylisopropyl)adenosine (PIA), and of the methylxanthine, theophylline, were determined in 19 vagotomized glomectomized cats whose end-tidal PCO2 was kept constant by means of a servocontrolled ventilator. Integrated phrenic nerve activity was used to represent respiratory output. Our results show that PIA, whether given systemically or into the third cerebral ventricle, depressed respiration. Systemically administered theophylline stimulated respiration. Theophylline given intravenously, or into the third ventricle not only reversed the depressive effects of previously administered PIA but caused further increases of respiration above the control level. Prior systemic administration of theophylline blocked both respiratory and hypotensive effects of subsequently administered PIA. Effects of either agent on medullary extracellular fluid pH did not explain the results. We conclude that the adenosine analogue PIA, acts to inhibit neurons in the brain that are involved in the control of respiration and that its effects are blocked by the ophylline. We suggest that adenosine acts as a tonic modulator of respiration and that theophylline stimulates breathing by competitive antagonism of adenosine at neuronal receptor sites.
- Eldridge FL, Millhorn DE, Waldrop TG and Kiley JP (1983). Mechanism of respiratory effects of methylxanthines. Respir Physiol **53**:239-61. Summary: Neural

respiratory responses to the ophylline, aminophylline and ethylenediamine were determined in paralyzed, vagotomized and glomectomized cats whose end-tidal PCO2 and brain temperature were kept constant. Intravenous theophylline and aminophylline similarly stimulated respiration, but ethylenediamine had no effect. The following did not cause the response: muscular and mechanical factors, carotid body and vagal reflexes, spinally mediated mechanisms arising below C7, changes of arterial PCO2 or medullary ECF pH, changes of whole body metabolic rate or release of substances from the adrenal glands. Absence of supraportine brain did not prevent the response. Pretreatment with a serotonin antagonist did not affect the response but two different dopamine antagonists caused its attenuation. When administered into the third ventricle, theophylline did not stimulate respiration, but both aminophylline and ethylenediamine, due to the latter's ability to mimic the inhibitory effects on neurons of gamma-aminobutyric acid (GABA), caused significant depression of respiration. We conclude that the neural respiratory response to systemically administered theophylline is mediated at the level of the brainstem, and somehow involves the action of the neurochemical dopamine. The failure of cerebroventricularly administered theophylline to stimulate respiration must be related to its inability to reach the appropriate neurons from the cerebrospinal fluid. Fujii S, Kato H, Ito K, Itoh S, Yamazaki Y, Sasaki H and Kuroda Y (2000). Effects of A1 and A2 adenosine receptor antagonists on the induction and reversal of long-term potentiation in guinea pig hippocampal slices of CA1 neurons. Cell Mol Neurobiol 20:331-50. Summary: 1. Using simultaneous recordings of the field EPSP and the population spike in the CA1 neurons of guinea pig hippocampal slices, we confirmed that delivery of a high-frequency stimulation (tetanus: 100 pulses at 100 Hz) produced robust long-term potentiation of synaptic efficacy (LTP) in two independent components, a synaptic component that increases field excitatory postsynaptic potentials (EPSPs) and a component that results in a larger population spike amplitude for a given EPSP size (E-S potentiation). 2. In the same cells, reversal of LTP (depotentiation; DP) in the field EPSP and in the E-S component is achieved by delivering low-frequency afferent stimulation (LFS: 1 Hz, 1000 pulses) 20 min after the tetanus. 3. When the tetanus or LFS was applied to CA1 inputs in the presence of an adenosine A1 receptor antagonist, 8cyclopentyltheophylline (1 microM), the field EPSP was enhances in LTP and attenuated in DP, while the E-S relationship was not significantly affected in either LTP or DP. 4. When similar experiments were performed using an A2 receptor antagonist, CP-66713 (10 microM), the field EPSP was blocked in LTP but facilitated in DP, while E-S potentiation was enhanced during both LTP and DP. 5. The results show that endogenous adenosine, acting via A1 or A2 receptors, modulates both the synaptic and the E-S components of the induction and reversal of LTP. Based on the results, we discuss the key issue of the contribution of these receptors to the dynamics of neuronal plasticity modification in hippocampal CA1 neurons. Department of Physiology, Yamagata University School of Medicine, Japan. sfujii@med.id.yamagata-u.ac.jp

• Huang LT, Liou CW, Yang SN, Lai MC, Hung PL, Wang TJ, Cheng SC and Wu CL (2002). Aminophylline aggravates long-term morphological and cognitive damages in status epilepticus in immature rats. Neurosci Lett **321**:137-40. Summary: Here, we investigated whether aminophylline, an adenosine receptor antagonist used usually as a treatment for premature apnea, had synergistic effects on status epilepticus in the developing brain. On postnatal day 14 (P14), four groups of rats intraperitoneally

received saline, aminophylline, lithium--pilocarpine (Li-PC), and Li-PC plus aminophylline, respectively. Subsequently, the Morris water maze task was performed at P80. The brains were then analyzed with cresyl violet stain for histological lesions and evaluated for mossy fiber sprouting with the Timm stain. No seizures were elicited in the saline-treated or aminophylline-treated rats. Both the Li-PC-treated and aminophylline plus Li-PC-treated rats exhibited seizures and there was no significant difference in mortality between the two groups. More interestingly, as in adulthood (P80), aminophylline aggravated the spatial deficits and histological damages seen in Li-PC-treated rats. In summary, this present study suggests that the use of adenosine receptor antagonists, such as aminophylline, exacerbates seizure-induced damage in the developing brain. Department of Pediatrics, Chang Gung Memorial Hospital, 123, Ta-Pei Road, Niao-Sung, Kaohsiung, Taiwan. huang_li@taiwan.com

- Kawai A, Okada Y, Muckenhoff K and Scheid P (1995). Theophylline and hypoxic ventilatory response in the rat isolated brainstem-spinal cord. Respir Physiol 100:25-32. Summary: We have used the isolated brainstem-spinal cord preparation of the neonatal rat to study the effects of the ophylline on the ventilatory response to hypoxia. The brainstem-spinal cord was isolated from neonatal rats (0-4 days) and superfused with mock cerebrospinal fluid (CSF), equilibrated with a gas mixture (FO2, 0.90; FCO2, 0.02; FN2, 0.08; control CSF) at 27 degrees C. We recorded phrenic nerve discharge from C4 roots, using suction electrodes, and measured respiratory frequency (fR) and the amplitude of the integrated phrenic neurogram (integral of phr). We examined how theophylline and the specific adenosine antagonist, 8-p-sulfophenyltheophylline (SPT), modify the ventilatory response to hypoxia. The response during superfusion with hypoxic CSF (FO2, 0.06) consisted of a marked decrease in fR (to 60% of control) and a slight decline in integral of phr (to 85% of control). By contrast, in the presence of theophylline (30 mg/L = 165 microM) and SPT (5 mg/L = 15 microM) in the superfusate hypoxia reduced fR only moderately (to 87% of control) and exerted virtually no effect on integral of phr (105% of control). The ophylline and SPT attenuated the rate of decrease in fR and completely blocked the decrease in integral of phr. There was no difference between the effects of the ophylline and those of SPT. The results suggest that theophylline attenuates hypoxic respiratory depression, and that this effect is mediated by the blockade of adenosine. Institut fur Physiologie, Ruhr-Universitat Bochum, Germany.
- Krottmayer G, Kerbel R, Muller WD and Kurz R (1985). [Congenital central hypoventilation syndrome--loss of chemosensitivity in respiratory control]. Monatsschr Kinderheilkd 133:764-6. Summary: This report describes an infant with congenital central hypoventilation. There is no response to 4% CO2-breathing in sleep and in awake state. Hypoxia, behavioral and "behavioral like" inputs increase ventilation, but not to normal levels. Drugs such as theophylline, naloxone, acetazolamide, methylprogesterone, thyroxine and nicethamide have no effect on the respiratory control. Despite the insertion of a phrenic nerve pacemaker intermittent positive pressure ventilation must be provided in addition.
- Masino SA and Dunwiddie TV (2000). A transient increase in temperature induces persistent potentiation of synaptic transmission in rat hippocampal slices. Neuroscience **101**:907-12. Summary: Previous studies have shown that increasing the temperature of rat hippocampal brain slices from 32.5 to 38.5 degrees C initiates a profound, adenosine-mediated decrease in excitatory synaptic transmission in the CA1 region. Here we found

that upon lowering the temperature back to 32.5 degrees C, the amplitude of the field excitatory postsynaptic potential often recovers to a level that is significantly potentiated with respect to the initial baseline. This potentiation is rapid in onset (< 5min following return to 32.5 degrees C) and long lasting (>60min following the termination of the increase in temperature). Similar effects could not be induced by superfusion with adenosine alone, and adenosine receptor antagonists did not block the potentiation. Therefore, although an adenosine-mediated decrease in excitatory synaptic transmission occurs during the temperature increase, it is unrelated to the potentiation. Likewise, N-methyl-D-aspartate receptor activation is not required, as N-methyl-D-aspartate receptor antagonists do not influence this form of potentiation. In summary, we propose that transiently increasing brain slice temperature represents a novel way to induce synaptic plasticity in the hippocampus, and may provide a paradigm to elucidate additional cellular mechanisms involved in functional plasticity. Department of Pharmacology and Neuroscience Program, University of Colorado Health Sciences Center, Denver, CO 80262, USA. susan.masino@uchsc.edu

- Millhorn DE, Eldridge FL, Kiley JP and Waldrop TG (1984). Prolonged inhibition of respiration following acute hypoxia in glomectomized cats. Respir Physiol **57**:331-40. Summary: Respiratory responses to several minutes exposure to hypoxia (PaO2 less than 30 torr) were determined in anesthetized, paralyzed, vagotomized and glomectomized cats whose end-tidal PCO2 and body temperature were kept constant. Respiratory activity was quantified from phrenic nerve activity. Animals breathed 100% O2 during the control period. The study reaffirmed that in glomectomized animals hypoxia causes depression of respiratory activity. The new finding was that phrenic activity remained significantly depressed below the original control level for more than one hr after return to the hyperoxic state. Medullary ECF pH was measured in 3 cats. There was an acid shift of pH during hypoxia that persisted for more than one hour after return to hyperoxic state. We pretreated another group (n = 5) of animals with the ophylline, a specific antagonist of the inhibitory neurotransmitter adenosine. Hypoxia still caused depression of respiratory activity, but it was less severe than in untreated animals. Upon return to the hyperoxic state, respiratory activity returned to the original control level within 10 min. We conclude that the long-lasting depression of respiration following hypoxia is mediated by adenosine. Furthermore, adenosine appears to be partially responsible for the acute depression of respiration during the hypoxic exposure.
- Nantwi KD, El-Bohy A and Goshgarian HG (1996). Actions of systemic theophylline on hemidiaphragmatic recovery in rats following cervical spinal cord hemisection. Exp Neurol 140:53-9. Summary: This study assesses the effects of theophylline on enhancing phrenic nerve discharge and functional hemidiaphragmatic recovery after C2 spinal cord hemisection in adult female rats. There were three separate groups of spinal hemisected rats and one nonhemisected group studied. Twenty-four hours following C2 spinal hemisection, ipsilateral phrenic nerve activity was recorded under standardized, normoxic and then hypoxic conditions. After 30 min, theophylline was administered and the recordings were repeated in group 1 animals. In group 2, activity in both phrenic nerves was recorded simultaneously before and after drug administration. In a third group of rats, both ipsilateral phrenic nerve and hemidiaphragmatic activities were monitored before and after the drug. In control nonhemisected animals under standardized recording conditions, the effects of theophylline were quantitatively assessed by determining the

mean area under integrated phrenic nerve discharge waveforms before and after drug administration. Generally, theophylline induced biphasic effects; i.e., at a low dose (15 mg/kg) it evoked excitation, while at a high dose (30 mg/kg) depression of respiratory activity predominated. In group 2 animals, respiratory activity was induced in the nerve ipsilateral to the hemisection and enhanced in the contralateral phrenic nerve for up to 3 h after a single standard dose of theophylline (15 mg/kg). Prior to drug administration, there was an absence of respiratory-related activity in both the phrenic nerve and hemidiaphragm ipsilateral to C2 spinal cord hemisection. A standard dose of theophylline, however, induced recovery of activity in both the phrenic nerve and the left hemidiaphragm ipsilateral to the hemisection in group 3 animals. In control (nonhemisected) animals, the ophylline enhanced phrenic nerve activity, but decreased the duration of respiratory bursts. These results show for the first time that theophylline can activate latent respiratory motor pathways and thus restore the respiratory drive to phrenic motoneurons lost by spinal cord injury. Respiratory activity is not only reestablished in the phrenic nerve made quiescent by hemisection, but it is also enhanced in the contralateral phrenic nerve. The drug also restores function to the hemidiaphragm paralyzed by the spinal cord hemisection. The findings may have clinical relevance to human cases of cervical spinal cord injury in which respiratory function is compromised. Department of Anatomy and Cell Biology, Wayne State University, School of Medicine, Detroit, Michigan 48201, USA.

- Nantwi KD and Goshgarian HG (1998a). Effects of chronic systemic theophylline injections on recovery of hemidiaphragmatic function after cervical spinal cord injury in adult rats. Brain Res **789**:126-9. Summary: Based on a previous demonstration that acutely administered theophylline induces respiratory-related recovery in an animal model of spinal cord injury, the influence of chronically administered theophylline on maintaining recovery was assessed. The absence of respiratory-related activity in the left phrenic nerve and hemidiaphragm of rats subjected to an ipsilateral C2 spinal cord hemisection was confirmed electrophysiologically 24 h after injury. Theophylline was then injected i.p. for 3-30 consecutive days. Recovery of respiratory-related activity was observed in the majority (29 out of 32) of the experimental animals. We conclude that theophylline not only induces, but also maintains recovery for prolonged periods after cervical spinal cord injury. Department of Anatomy and Cell Biology, Wayne State University, Detroit, MI 48201, USA. knantwi@med.wayne.edu
- Nantwi KD and Goshgarian HG (1998b). Theophylline-induced recovery in a hemidiaphragm paralyzed by hemisection in rats: contribution of adenosine receptors. Neuropharmacology **37**:113-21. Summary: Previously, we demonstrated that a single intravenous injection of theophylline can induce recovery in a hemidiaphragm paralyzed by cervical (C2) spinal cord hemisection for up to 3 h. The present study contrasts the actions of enprofylline and theophylline on inducing hemidiaphragmatic recovery after cervical spinal cord hemisection. Both drugs are methylxanthines; however, theophylline is an adenosine receptor antagonist while enprofylline is not. To further test the involvement of adenosine receptors, N6 (L-2-phenylisopropyl) adenosine (L-PIA), an analogue of adenosine was used in conjunction with theophylline. Following a left C2 spinal cord hemisection, animals were injected with either enprofylline (2.5-20 mg/kg) or theophylline (15 mg/kg) alone or in combination. Theophylline-injected animals demonstrated robust respiratory-related activity in the previously quiescent left phrenic

- nerve and hemidiaphragm. No recovery was observed in any of the enprofylline-injected rats. When enprofylline injection was followed later with theophylline, recovery occurred. Prior L-PIA administration blocked theophylline-induced recovery. When given after theophylline, L-PIA attenuated and then blocked the induced activity in both the nerve and hemidiaphragm ipsilateral to spinal cord hemisection. We conclude that adenosine receptor antagonism is implicated in hemidiaphragmatic recovery after hemisection and theophylline may be useful in the treatment of spinal cord injured patients with respiratory deficits. Wayne State University, School of Medicine, Department of Anatomy and Cell Biology, Detroit, MI 48201, USA.
- Nantwi KD and Goshgarian HG (2001). Alkylxanthine-induced recovery of respiratory function following cervical spinal cord injury in adult rats. Exp Neurol 168:123-34. Summary: Previous investigations from our laboratory have demonstrated qualitatively that a latent respiratory pathway can be activated by systemic theophylline administration to restore function to a hemidiaphragm paralyzed by an upper (C2) cervical spinal cord hemisection in adult rats. The present study seeks to extend the previous investigations by contrasting and quantitating the actions of theophylline, 8-phenyltheophylline, enprofylline, and 8(p-Sulfophenyl)theophylline in restoring function 24 h after hemidiaphragm paralysis. The alkylxanthines were selected based on their diverse pharmacologic profiles to elucidate the mechanisms that underlie functional recovery after spinal cord injury. To quantitatively assess the magnitude of recovery, electrophysiological experiments were conducted on pancuronium-paralyzed, hemisected animals under standardized recording conditions. The total absence of respiratory-related activity in the phrenic nerve ipsilateral to the hemisection and paralyzed hemidiaphragm was used as the index of a functionally complete hemisection. Thereafter, drug-induced recovered activity in the phrenic nerve ipsilateral to hemisection was quantified and expressed either as a percentage of contralateral phrenic nerve activity in the same animal prior to drug administration or as a percentage of predrug activity in the homolateral nerve in noninjured animals. With either approach, theophylline (5-15 mg/kg) and 8phenyltheophylline (5-10 mg/kg) dose-dependently induced respiratory-related recovered activity. Enprofylline, a potent bronchodilator, and 8(p-Sulfophenyl)theophylline, an adenosine receptor antagonist with limited access to the central nervous system, were ineffective. Maximal recovery was attained with theophylline (15 mg/kg) and 8phenyltheophylline (10 mg/kg). At these doses, theophylline and 8-phenyltheophylline induced recovery that was 70.0 +/- 2.5 and 69.3 +/- 4.1% of predrug contralateral nerve activity respectively. When expressed as a percentage of activity in the homolateral nerve in noninjured animals, the magnitude changed to 32.9 +/- 4.9 and 35.7 +/- 6.9%, respectively. Involvement of adenosine receptors in the alkylxanthine-induced actions was confirmed in experiments with the adenosine analog, N6 (1-2-phenylisopropyl) adenosine (L-PIA). It is concluded that central adenosine receptor-mediated mechanisms are implicated in the recovery of respiratory-related activity after spinal cord injury. Furthermore, our results suggest a potential for a new therapeutic approach in the rehabilitation of spinal cord patients with respiratory deficits. Department of Anatomy and Cell Biology, Wayne State University, School of Medicine, 540 East Canfield Avenue, Detroit, Michigan 48201, USA.
- Nantwi KD and Goshgarian HG (2002). Actions of specific adenosine receptor A1 and A2 agonists and antagonists in recovery of phrenic motor output following upper cervical

spinal cord injury in adult rats. Clin Exp Pharmacol Physiol **29**:915-23. Summary: 1. Previous studies from our laboratory have established that a latent respiratory motor pathway can be activated to restore function to a hemidiaphragm paralysed by upper cervical (C2) spinal cord hemisection during a reflex known as the 'crossed phrenic phenomenon'. In addition, theophylline, a general adenosine A1 and A2 receptor antagonist, can activate the latent pathway by acting centrally through antagonism at adenosine receptors. 2. The present study was designed to assess the relative contributions of adenosine A1 and A2 receptors in inducing functional recovery in our model of spinal cord injury. Specific adenosine A1 and A2 agonists and antagonists were used in an electrophysiological study. 3. Our results demonstrate that, in hemisected rats, systemic administration of the adenosine A1 receptor-specific antagonist 1,3-dipropyl-8cyclopentylxanthine (DPCPX) restores, in a dose-dependent manner, phrenic nerve respiratory related output that is lost following hemisection. Furthermore, DPCPX augments respiratory activity in non-injured animals. The A2 receptor agonist CGS-21680 mediates its effects by predominantly acting on peripheral rather than central nervous system (CNS) receptors. CGS-21680 modulates respiratory related phrenic nerve activity in non-injured animals by enhancing tonic activity, but does not induce recovery of phrenic nerve activity in hemisected animals in the majority of cases. When CGS-21680 was administered prior to DPCPX in hemisected rats, the magnitude of recovery of respiratory function was significantly greater than that elicited by DPCPX alone. However, when the A2 receptor agonist was administered after DPCPX, the magnitude of recovery was virtually unchanged, whereas activity in the right phrenic nerve was significantly enhanced. The A1 receptor agonist N6-cyclohexyladenosine depressed respiratory activity in non-injured, as well as hemisected, rats. The A2 receptor antagonist 3,7-dimethyl-1-propargylxanthine did not affect respiratory activity. 4. We conclude that while antagonism at central adenosine A1 receptors mediates functional restitution in hemisected animals, activation of A2 receptors located outside of the CNS subserves the A1 receptor-mediated respiratory recovery. Wayne State University, School of Medicine, Department of Anatomy and Cell Biology, Detroit, Michigan 48201, USA. knantwi@med.wayne.edu

• Qiu J, Cai D, Dai H, McAtee M, Hoffman PN, Bregman BS and Filbin MT (2002a). Spinal axon regeneration induced by elevation of cyclic AMP. Neuron 34:895-903. Summary: Myelin inhibitors, including MAG, are major impediments to CNS regeneration. However, CNS axons of DRGs regenerate if the peripheral branch of these neurons is lesioned first. We show that 1 day post-peripheral-lesion, DRG-cAMP levels triple and MAG/myelin no longer inhibit growth, an effect that is PKA dependent. By 1 week post-lesion, DRG-cAMP returns to control, but growth on MAG/myelin improves and is now PKA independent. Inhibiting PKA in vivo blocks the post-lesion growth on MAG/myelin at 1 day and attenuates it at 1 week. Alone, injection of db-cAMP into the DRG mimics completely a conditioning lesion as DRGs grow on MAG/myelin, initially, in a PKA-dependent manner that becomes PKA independent. Importantly, DRG injection of db-cAMP results in extensive regeneration of dorsal column axons lesioned 1 week later. These results may be relevant to developing therapies for spinal cord injury. Biology Department, Hunter College, City University of New York, 695 Park Avenue, New York, NY 10021, USA.

- Qiu J, Cai D and Filbin MT (2000). Glial inhibition of nerve regeneration in the mature mammalian CNS. Glia 29:166-74. Summary: The lack of axonal regeneration in the adult mammalian CNS is due to both unfavorable environmental glial factors and the intrinsic neuronal state. Inhibitors associated with myelin and the glial scar have been extensively studies and it has been shown that neutralizing at least some of the inhibitors can lead to improved growth. Meanwhile, important advances have also been made towards our understanding of the neuronal intrinsic state, particularly the intracellular levels of cyclic nucleotide, that influence the capacity of mature CNS neurons to initiate and maintain a regrowth response. It is well recognized that successful regeneration may only be achieved by application of a combination of strategies that both block glial inhibitors and enhance the intrinsic neuronal growth capacity. Biology Department, Hunter College of CUNY, New York, New York 10021, USA.
- Qiu J, Cai D and Filbin MT (2002b). A role for cAMP in regeneration during development and after injury. Prog Brain Res **137**:381-7. Summary: Biology Department, Hunter College, City University of New York, 695 Park Avenue, New York, NY 10021, USA.
- Storozhuk MV and Balaban PM (1989). [The role of cyclic adenosine monophosphate in simple forms of plasticity in the edible snail]. Zh Vyssh Nerv Deiat Im I P Pavlova 39:543-7. Summary: cAMP-dependence of synaptic depression and facilitation was investigated in functionally identified synaptic connection in the snail. It was found that 5 mM imidazole (phosphodiesterase activator) as well as 2 mM tolbutamide (inhibitor of cAMP-dependent protein kinase) do not change the rate of EPSPs depression during rhythmic (0.1 Hz) nerve stimulation, and do not affect facilitation. But treatment with both these drugs decreases EPSPs amplitude. Possibility of cAMP-dependent modulation of synaptic effectiveness is discussed.
- Thummler S and Dunwiddie TV (2000). Adenosine receptor antagonists induce persistent bursting in the rat hippocampal CA3 region via an NMDA receptor-dependent mechanism. J Neurophysiol 83:1787-95. Summary: Adenosine receptor antagonists initiate repetitive bursting activity in the CA3 region of hippocampal slices. Although some studies have suggested that this effect is irreversible, this has been difficult to establish because many adenosine antagonists wash out of brain slices extremely slowly. Furthermore the cellular mechanism that underlies persistent bursting is unknown. To resolve these issues, we studied the effects of nonselective (8-p-sulfophenyltheophylline, 8SPT, 50-100 microM), A(1)-selective (8-cyclopentyl-1, 3-dipropylxanthine, 100 nM; xanthine carboxylic acid congener, 200 nM), and A(2A)-selective (chlorostyryl-caffeine; 200 nM) adenosine antagonists in the CA3 region of rat hippocampal slices using extracellular recording. Superfusion with all of the adenosine antagonists except chlorostyryl-caffeine induced bursting, and the burst frequency after 30 min drug superfusion did not differ for the different antagonists. Most slices showed a period of rapid initial bursting, followed either by stable bursting at a lower frequency or a pattern of oscillating burst frequency. In either case, the bursting continued after drug washout. Virtually identical patterns of long-term bursting activity were observed when 8SPT was washed out or applied continuously. Control experiments using exogenous adenosine to characterize the persistence of 8SPT in tissue demonstrated >95% washout at 60 min, a time when nearly all slices still showed regular bursting activity. When the N-methyl-Daspartate (NMDA) antagonists DL-2-amino-5-phosphonovaleric acid (AP5; 50 microM)

or dizocilpine (10 microM) were applied before and during 8SPT superfusion, bursting occurred in the presence of the NMDA antagonists but did not persist once the 8SPT was washed out. AP5 had no effect on persistent bursting when applied after the initiation of spiking. The selective calcium/calmodulin-dependent protein kinase inhibitor 1-[N, O-bis-(5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine (KN-62; 3 microM), which has been shown to block NMDA receptor-dependent synaptic plasticity in the CA1 region, also significantly decreased the long-term effect of 8SPT. Thus adenosine antagonists initiate persistent spiking in the CA3 region; this activity does not depend on continued occupation of adenosine receptors by antagonists, and can be blocked by treatments that prevent NMDA receptor-dependent plasticity. Institut fur Pharmakologie und Toxikologie, Universitat Leipzig, D04107 Leipzig, Germany.