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Attempted Antagonism of Adenosine Analogue Induced Depression of Respiration

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MUELLER, R. A., E. WIDERLÖV AND G. R. BREESE. *Attempted antagonism of adenosine analogue induced depression of respiration.* PHARMACOL BIOCHEM BEHAV 21(2) 289–296, 1984.—Intracerebroventricular (ICV) administration of the stable adenosine analogue 2-chloroadenosine (2CA) to hyperoxic halothane-anesthetized rats produced a dose-dependent depression of respiration largely as a result of a decrease in tidal volume. Similar changes were noted after another adenosine analogue, phenylisopropyladenosine (PIA). Higher doses shifted the minute ventilation-$PaCO_2$ curve to the right and decreased its slope. Bradycardia and hypotension were produced at doses which altered respiration. Neonatal destruction of brain serotonin or dopamine-containing nerve terminals did not alter the 2CA-induced respiratory depression. Naloxone significantly antagonized the respiratory and circulatory changes produced by 2CA though the changes produced by PIA were not significantly antagonized. Peripherally and intracerebroventricularly administered theophylline were largely ineffective in reversing the 2CA-induced respiratory depression. Thus, these data suggest that a major part of the respiratory depression produced by 2CA is due to indirect activation of opioid receptors. In contrast, very little of the respiratory depression after PIA is via mechanisms antagonized by naloxone. Thus, putative adenosine agonists appear to vary in the extent to which respiratory depression is provoked by interactions with opioid systems.

Adenosine Respiration 2-Chloroadenosine Biogenic amines Anesthesia Phenylisopropyladenosine

AMINOPHYLLINE is being widely used for the management of neonatal apnea syndromes [7]. In this application, the frequency of apneic spells and their duration are decreased. Although a great deal is known about how aminophylline affects airway mechanics, we know very little of the mechanism responsible for its central stimulation of respiration. Neonatal destruction of central serotonergic neurones with pargyline and 5,7-dihydroxytryptamine potentiated the respiratory stimulation produced by aminophylline whereas similar destruction of brain stem dopaminergic neurones with desmethylimipramine and 6-hydroxydopamine reduced the magnitude of stimulation [32]. While the above data indicate the importance of biogenic amines in at least modulating the aminophylline-induced stimulation of respiration, the precise way in which aminophylline alters these biogenic amine-containing neurones is unknown.

Several authors have presented evidence that aminophylline may antagonize adenosine *in vitro* in isolated peripheral organs [2, 5 16]. In several pharmacological test systems, methylxanthines such as aminophylline have been shown to competitively antagonize the CNS effects of adenosine [37,42]. This antagonism may be visible at concentrations of aminophylline below those which alter calcium dynamics or phosphodiesterase mediated inactivation of cAMP [8,37]. Thus, the respiratory stimulation observed after aminophylline may be due to antagonism of endogenous adenosine which might in turn alter biogenic amine neuronal activity. The present investigation sought to examine the respiratory effects of a stable adenosine analogue, 2-chloroadenosine (2CA), and to determine whether aminophylline would reverse these changes. In addition, because some similarities have been noted between the peripheral tissue responses to purines and the musculotropic actions of narcotics [16], a narcotic antagonist, naloxone, was also assessed for its ability to antagonize the respiratory depression produced by 2CA and phenylisopropyladenosine (PIA), another metabolically stable analogue of adenosine. Preliminary reports of these studies were presented elsewhere [34,35].

METHOD

Measurement of Respiration in Anesthetized Rats

Sprague-Dawley rats of either sex, weighing 200–300 g were used. Under ether anesthesia, animals had guide cannulae aimed at the lateral cerebral ventricle and cemented to the skull at least 48 hours before use. On the day of study, the rats were lightly anesthetized with ether to permit placement of cannulae in the tail or femoral artery (to monitor heart rate, blood pressure and blood gas tensions) and the trachea. An inner cannula (ICV cannula) of length sufficient to reach the ventricle space was then inserted through the guide. All ventricular placements were verified by dye injection at the conclusion of the respiratory studies, and only animals with staining of the floor of the fourth ventricle were included in the data analysis. Following all surgical procedures, the animal was given 0.7% halothane in oxygen to breathe and placed in a closed body plethysmograph to permit recording of respiratory tidal volume and frequency

TABLE 1
RESPIRATORY DOSE RESPONSE FOR 2-CHLOROADENOSINE

	Tidal Volume ml/100 g		Min Ventilation ml/100 g/min	
	15 min	30 min	15 min	30 min
1.67 μg	0.48 ± 0.05 (86)	0.55 ± 0.06 (96)	51.1 ± 6.4 (97)	52 ± 6.4 (99)
5 μg	0.45 ± 0.02* (80)	0.48 ± 0.03 (85)	40.4 ± 2.2* (78)	45 ± 1.8* (85)
16.7 μg	0.39 ± 0.03* (70)	0.43 ± 0.03 (77)	34 ± 4.5† (66)	34 ± 0.9† (64)

Values represent the mean ± SEM of 5–6 rats. Numbers in parentheses are as % of corresponding value before drug addition.
*$p<0.05$; †$p<0.01$ compared to pre-drug values.

[26,33]. Body temperature was monitored with a rectal probe and maintained constant with a heating pad. Though all anesthetics are known to alter respiratory drive, as well as the mechanics of respiration [29], control animals received the same concentrations of halothane as did rats given the test drug; thus, responses are presumed to be due to the test drug.

Experiments started with a stabilization period of 20 minutes, during which the rat was breathing 0.7% halothane 99.3% O_2. This concentration of halothane was always included in all subsequent inhaled gas mixtures. Following the stabilization period, responsiveness to CO_2 was tested using mixtures of 2.5 and 5% CO_2 in O_2 and halothane. Each gas was given for 5 minutes, at which point mechanical changes in respiration had been stable for at least 2 to 3 minutes. Separate, parallel experiments in anesthetized rats revealed little further change in $PaCO_2$ if a longer equilibrium time was allowed. After a further 10 minutes on O_2, when the respiratory parameters had returned to near pre-CO_2-exposure values, the drug under study was given via the ICV cannula. Each rat received only one dose of any drug. At 5-minute intervals after drug administration (see "Results"), measurements of blood pressure, heart rate, respiratory rate, and tidal volume were made. After the 15-minute values were recorded, the two above sequential 5-minute CO_2 exposures were repeated. At the end of recording the 5% CO_2 response animals were given oxygen and halothane for 5 minutes and measurements repeated (30 minutes after drug). The plethysmograph was calibrated at the end of studies on each rat as previously described [33].

Blood Gas Measurement

At the termination of each inspired gas mixture (initial 20 minutes, after 2½% CO_2, 5% CO_2, 10 minutes oxygen, 15 minutes after drug, after 2½% CO_2, 5% CO_2, and just before pancuronium administration), 0.3 ml of arterial blood was withdrawn to permit determination of arterial pH, CO_2 ($PaCO_2$) and O_2 ($PaCO_2$) tensions using a Radiometer MBS3 MK2 Blood Micro System blood analyzer. Immediately after withdrawal of each of these 8 blood samples, an equivalent volume of saline (0.9%) was injected slowly IA. Removal and replacement of these blood volumes over 50 minutes (as described above) did not alter cardiovascular or respiratory parameters in animals given saline ICV.

Selective Lesions of Brain Serotonin-Containing Neurones

In order to decrease the number of serotonin-containing nerve terminals, 3-day-old rats were given 50 μg (ICV) of 5,7-dihydroxytryptamine (5,7-DHT) creatinine sulfate 30 minutes after desmethylimipramine (DMI) (20 mg/kg IP) [3]. Animals were deprived of a majority of central dopamine-containing neurones by receiving 100 μg 6-hydroxydopamine (6-OHDA) IC one hour after 20 mg/kg DMI at 5 days of age [4].

Drugs

Doses of all drugs are expressed as the salts. The 2-cholroadenosine was obtained from Sigma Chemical Co., St. Louis, Phenylisopropyladenosine [(−)-N^6-(R-Phenylisopropyl)-Adenosine] was purchased from Boehringer-Mannheim, Indianpolis, IN. The 5,7-dihydroxytryptamine creatinine sulfate and 6-hydroxydopamine hydrobromide were purchased from Regis Chemicals, Chicago, IL. Pargyline hydrochloride was purchased from Saber Laboratories, Inc., Morton Grove, IL, and desmethylimipramine hydrochloride was kindly supplied by the US Vitamin Pharmaceutical Corp. (Tuckahoe, NY). Aminophylline used in the intravenous infusion was purchased from Ivenex (Chagrin Falls, OH), whereas the theophylline used in ICV injections was purchased from Calbiochem (San Diego, CA) dissolved in warm water-saline to maintain isotonicity and adjusted to pH 7.35. Naloxone hydrochloride was supplied by Endo Laboratories (Garden City, NY).

Statistics

Statistical analyses employed Student's *t*-test (paired and unpaired) or an analysis of variance using Tukey's w procedure to assess significance between groups [39].

RESULTS

Changes in Ventillation after 2CA

A dose of 5 μg 2CA ICV produced a decrease in minute ventilation, largely a result of a decrease in tidal volume which developed within 1 minute and was maximal at 15 minutes (Table 1, Fig. 1). Doses of 50 μg or greater of 2CA produced apnea. $PaCO_2$ was significantly elevated ($p<0.01$) at both 15 and 30 minutes after drug administration (Fig. 1).

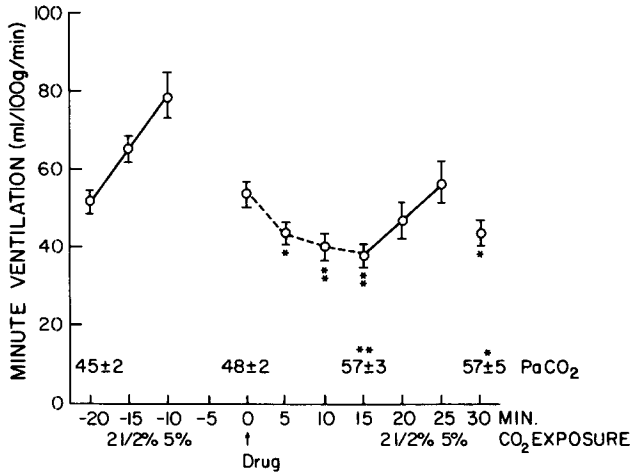

FIG. 1. Effect of 2CA on respiration. Each point represents the mean±SEM (brackets) of 5–6 rats. Each rat received 5 μg 2CA/5λ ICV at arrow. Numbers above horizontal axis are $PaCO_2$ in mmHg. Zero time is 20 min after start of administration of 0.7% halothane in O_2. $PaCO_2$ and minute ventilation values immediately before and during $2^1/_2$ or 5% CO_2 administration before and after drug administration appear in Fig. 2. $^{**}p<0.01$, $^{*}p<0.05$ relative to corresponding value immediately before drug addition.

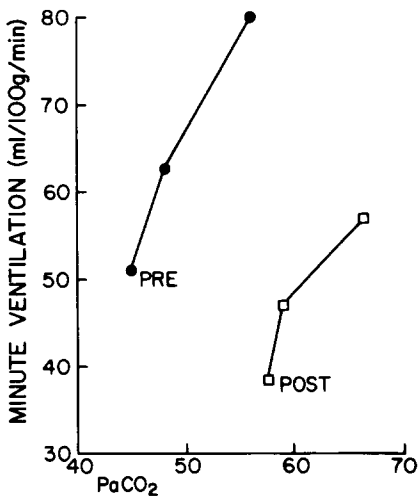

FIG. 2. Effect of 2CA on minute ventilation-$PaCO_2$ response curve. Pre-drug values (solid) were obtained while rats were breathing 0.7% halothane in O_2 only for 20 minutes, $2^1/_2$% CO_2 for 5 minutes and 5% CO_2 for 5 minutes, whereas post-drug values (open) were obtained 15–25 minutes after ICV 2CA (see Fig. 1 and the "Method" section for sequence of inhalational mixture exposure). All values for post-drug $PaCO_2$ and minute ventilation were significantly different from the corresponding pre-drug values. The slopes of the least squares linear regression equation for each of the two groups of points were not significantly different from each other.

Though 1.67 μg 2CA did not significantly alter minute ventilation (Table 1), the decrease observed at the 16.7 μg dose was greater than that observed at 5 μg. The minute ventilation-$PaCO_2$ response curve obtained 15–25 minutes after drug was shifted to the right at the 5 μg dose (Fig. 2), and in addition, the slope decreased significantly ($p<0.05$) at the 16.7 μg dose (see Fig. 8). Pulse rate was relatively more affected with 5 μg of 2CA than was blood pressure, but both were significantly reduced at doses similar to those which altered respiration, and remained depressed throughout the 30-minute observation period (Fig. 3).

Effect of Neonatal Cytotoxic Removal of Serotonin-Containing or Dopamine-Containing Neurones on the Respiratory Response to 2CA

Rats given 5,7-DHT at 3 days of age to reduce brain serotonin evidenced a decreased respiratory rate and minute ventilation when examined as adults. The $PaCO_2$ was elevated ($p<0.05$) in the 5,7-DHT-treated rats and the minute ventilation-$PaCO_2$ response curve was shifted to the right (Fig. 4). These findings are in agreement with earlier results [33]. Administration of 2CA to 5,7-DHT-treated rats decreased minute ventilation, increased $PaCO_2$ and shifted the minute ventilation-$PaCO_2$ curve as in animals given only DMI neonatally. The 5,7-DHT treatment decreased brain stem (pons + medulla) serotonin to 15±2% of control (DMI only) and 5HIAA to 18±3% of the concentration in the control group.

Neonatal DMI and 6-OHDA treatment reduced brain stem dopamine to 32±8% of control without significantly altering brain norepinephrine or serotonin. Nevertheless, the effects of 5 μg 2CA on minute ventilation and $PaCO_2$ (Fig. 5) were similar in degree to responses observed in control rats (see Fig. 1). Also, the minute ventilation-$PaCO_2$ curve was shifted similarly as noted in control rats (data not shown).

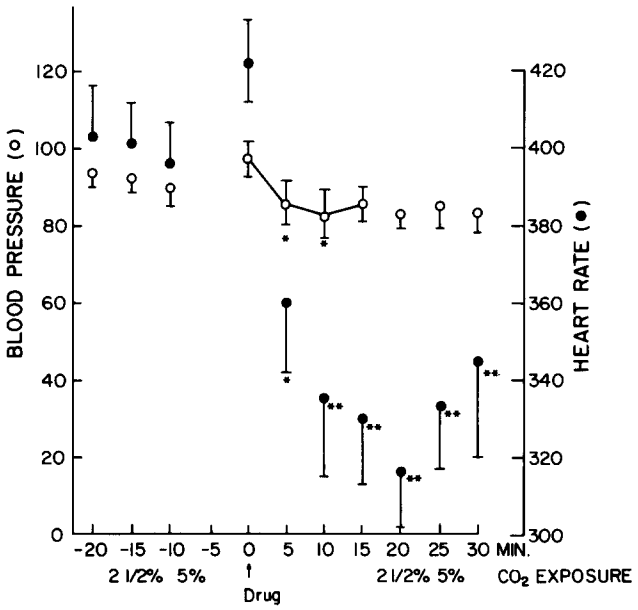

FIG. 3. Effect of 2CA on blood pressure and heart rate. Blood pressure and heart rate were recorded at the same time intervals as respiratory parameters in Figs. 1 and 2. Each value represents the mean±SEM (brackets) of 5–6 rats given 5 μg 2CA in 5λ CSF at arrow. $^{*}p<0.05$, $^{**}p<0.01$ relative to corresponding pre-drug value while breathing oxygen-halothane (at arrow) or pre-drug CO_2 concentration.

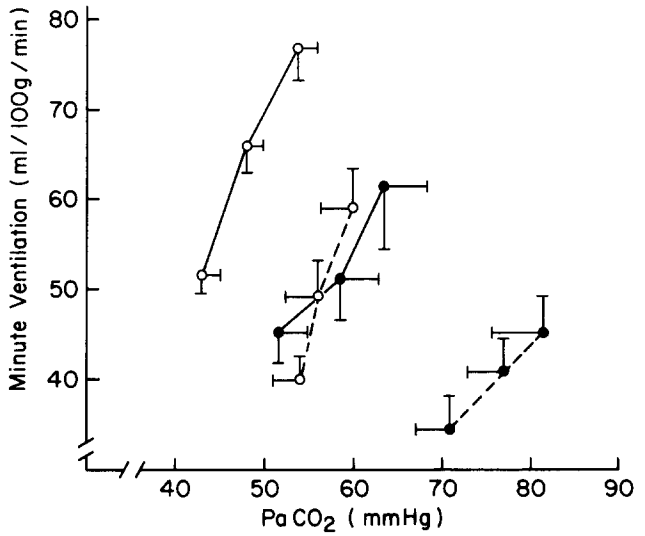

FIG. 4. Effect of 2CA on minute-ventilation-$PaCO_2$ response curve in control and 5,7-DHT treated rats. Control and 5,7-DHT rats were treated at 3 days of age with DMI (open circles) or DMI-5,7-DHT (closed circles) as described in the "Method" section. Respiratory baseline parameters when 250–300 g body weight were (control-5,7-DHT) frequency, 93±5–93±6 min^{-1}; tidal volume 0.57±0.02–0.48±0.01 ml/100 g; and minute ventilation, 52±1.8–45±3.7 ml/100 g/min. Neither 2CA nor neonatal 5,7-DHT treatment significantly altered the slope of the minute ventilation-$PaCO_2$ curve using the least squares method. Control rats (N=7) initial $PaCO_2$ (20 minutes on O_2) was 42.9±2.0 whereas 5,7-DHT rats (N=6) it was 51.4±3.8. The minute ventilation and $PaCO_2$ values for each point in the 5,7-DHT pre-drug curve (solid line) were significantly different ($p<0.05$) from those of the corresponding point in DMI pre-drug values (solid line). Similarly the minute ventilation and $PaCO_2$ values of post-drug points in both groups (dashed lines) were significantly different from the corresponding pre-drug values ($p<0.05$).

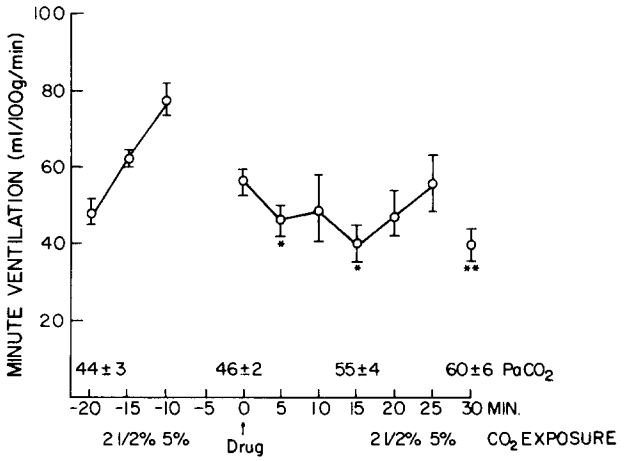

FIG. 5. Effect of 2CA on respiration in rats with deficient dopamine neurones. Each point represents the mean±SEM (brackets) of 6–7 rats treated neonatally with DMI and 6-hydroxydopamine as described in the "Method" section. Baseline respiratory parameters were: frequency=93±6.5 min^{-1}, tidal volume=0.53±0.03 ml/100 g. The changes in the minute ventilation-$PaCO_2$ curve after 2CA were similar to those of control animals (Fig. 2). Values above horizontal axis are the $PaCO_2$ values (mmHg) at indicated time intervals. *$p<0.05$, **$p<0.01$ relative to pre-drug control period.

Attempts to Antagonize the Depressant Effects of 2CA with Aminophylline

Initial studies attempted to antagonize the 2CA-induced respiratory depression with IV aminophylline infusions (Table 2). Previous studies with bolus IP injections of aminophylline revealed that 3 mg/kg significantly lowered $PaCO_2$ and increased minute ventilation [26]. Neither of the infusion schedules for aminophylline treatment used in the present study significantly altered respiratory parameters. Furthermore, the reduction of minute ventilation produced by 2CA and the absolute increase in $PaCO_2$ after 2CA was not significantly affected by 0.1 mg/kg/min aminophylline. In contrast, animals given 1 mg/kg/min aminophylline did not evidence a $PaCO_2$ statistically different from that present before 2CA administration, suggesting that at least one effect of 2CA was partially antagonized.

In the hope of finding a route of administration of theophylline which would more clearly antagonize the 2CA response, rats were given 100 μg theophylline ICV 5 minutes before the 2CA. Administration of 100 μg theophylline did not significantly alter cardiovascular or respiratory parameters (Fig. 6). Administration of 2CA 5 minutes later produced a similar depression of minute ventilation and an increase in $PaCO_2$ whether preceded by theophylline or saline (Fig. 6). Circulatory changes after 2CA were also unaltered by ICV theophylline. Administration of 50 μg

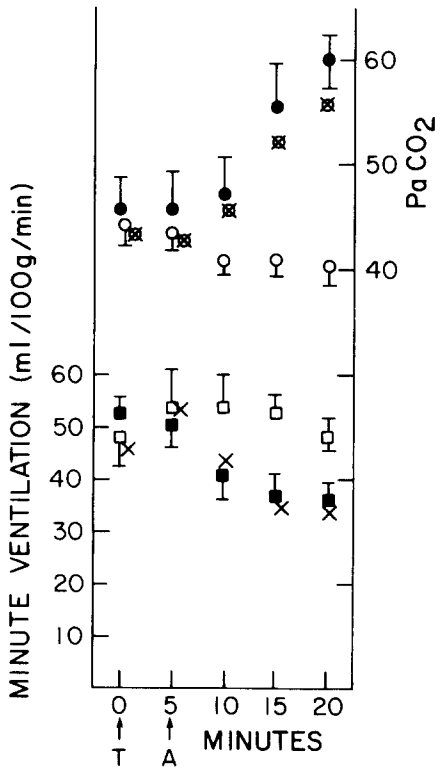

FIG. 6. Effect of intraventricularly-administered theophylline on the response to 2CA. Each point represents the mean±SEM (brackets) of 4–5 rats given 100 μg theophylline (open, cross symbols) or saline (solid symbols) at the arrow above "T". Five minutes later at the arrow over "A," they received either 5 μg 2CA (solid, cross symbols) or saline (open symbols). Respiratory and $PaCO_2$ values were then determined at 3 successive 5-minute periods. At ten and fifteen minutes after 2CA $PaCO_2$ was significantly elevated and minute ventilation significantly reduced ($p<0.05$) compared to values immediately before 2CA addition (solid and cross symbols).

TABLE 2

EFFECT OF AMINOPHYLLINE ON 2-CHLOROADENOSINE-INDUCED RESPIRATORY DEPRESSION

	Before 2CA			15 min Post 2CA		
	Resp. Rate resp/min	Minute Volume ml/100 g/min	$PaCO_2$ torr	Resp. Rate resp/min	Minute Volume ml/100 g/min	$PaCO_2$ torr
Control (8)[c]	95 ± 5.2	53 ± 2.3	46 ± 1.8	91 ± 6.3	40 ± 2.2*	54 ± 2.7* (+8)
0.1 mg/kg[a] (4)[c]	102 ± 12.6	48 ± 3.7	54 ± 2.1	98 ± 8.3	40 ± 1.7*	65 ± 4.2* (+11)
1 mg/kg[b] (5)[c]	107 ± 9.6	58 ± 3.7	40 ± 3.7	91 ± 6.0	40 ± 1.6*	46 ± 2 (+6)

Rats received aminophylline [a]0.1 mg/kg/min or [b]1 mg/kg/min intravenously for 30 min before and during 2-chloroadenosine administration (5 μg/5λ ICV). [c]Number of rats in each group.

*Values are significantly different from corresponding before 2CA values. Values in parentheses are the average increase in $PaCO_2$ in each group.

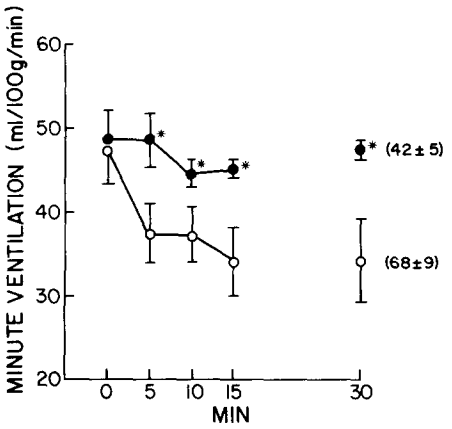

FIG. 7. Antagonism of the respiratory depressant effect of 2CA by naloxone. Each point represents the mean±SEM (brackets) of 7 rats. Control rats (open circles) were given 16.6 μg 2CA ICV at zero time (40 minutes after placement in the plethysmographic chamber (see the "Methods" section)). Naloxone treated rats (closed circles) received 10 μg naloxone/kg IA one minute before 2CA ICV. All ventilation values of naloxone/2CA rats were significantly greater (*$p<0.05$) relative to corresponding values for rats given only 2CA. Values on the far right are $PaCO_2$ of each group 30 minutes after 2CA (initial $PaCO_2$ values of both groups were 46±2.8 mmHg). At 20 and 25 minutes after 2CA administration animals were exposed to 2½% and 5% CO_2, respectively (see Fig. 8, and the "Method" section).

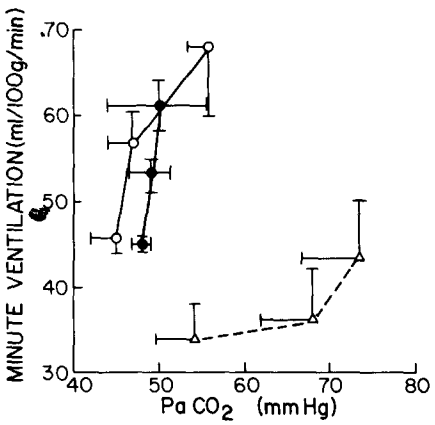

FIG. 8. Antagonism of 2CA-induced changes in the minute ventilation-$PaCO_2$ response curve by naloxone. Each value represents the mean±SEM (brackets) of 6–7 rats. Pre-drug values (open circles) were calculated using values of rats which subsequently received 2CA only or naloxone and 2CA (see the "Method" section). Post-drug values for rats given 2CA alone (open triangles) or naloxone and 2CA (solid circles) were obtained 15–25 minutes after 2CA administration (at 15, 20 and 25-minute points of Fig. 7) (see also the "Method" section). Using the least squares method the slope of the curve for rats given 2CA alone is significantly decreased ($p<0.01$), whereas that of naloxone plus 2CA rats were not significantly changed from the pre-drug values.

theophylline simultaneously with 2CA also did not alter the respiratory response (data not shown).

Effect of Naloxone on the Respiratory Response to 2CA and PIA

Naloxone (10 mg/kg via the femoral artery cannula) did not significantly affect circulatory parameters or the minute ventilation-$PaCO_2$ response curve in control rats nor was there a significant change in $PaCO_2$ or minute ventilation for the next 30 minutes (data not shown). However, after administration of this dose of naloxone one minute before 2CA, the minute ventilation response to 2CA (16.6 μg) was significantly antagonized, as was the change in $PaCO_2$ 30 minutes after 2CA (Fig. 7). In addition, the minute ventilation-$PaCO_2$ response curve was returned to the control position and slope by prior naxolone administration (Fig. 8). The bradycardia due to 2CA was significantly blunted but not abolished by naloxone treatment (Table 3). Administration of naloxone in the above fashion did not significantly blunt the respiratory depressant effects of PIA on the minute ventilation-$PaCO_2$ curve (Fig. 9), and higher naloxone doses did not further shift the curve toward the control position (data not shown).

DISCUSSION

The sedative and anticonvulsant properties of adenosine and various derivatives administered peripherally or into brain have been known for a long time [1,27]. More recent

TABLE 3

THE EFFECT OF NALOXONE ON THE CARDIOVASCULAR RESPONSE TO 2-CHLOROADENOSINE

Group	Time Interval: Minutes after 2-chloroadenosine				
	Control	5	10	15	30
Control					
BP	85 ± 3	88 ± 5	92 ± 4	93 ± 3	95 ± 8
HR	350 ± 10	330 ± 26	340 ± 24	350 ± 19	340 ± 11
2-Chloroadenosine					
BP	90 ± 3	72 ± 4	70 ± 6*	80 ± 7*	90 ± 4
HR	357 ± 22	300 ± 15	261 ± 21*	266 ± 24*	280 ± 23*
2-Chloroadenosine + Naloxone					
BP	88 ± 7	83 ± 3	75 ± 3*	75 ± 5*	83 ± 4
HR	360 ± 26	318 ± 13	305 ± 10‡	300 ± 8*	318 ± 9

Values were obtained from the same rats shown in Figs. 7 and 8. (Each value=mean ± SEM of 6 or 7 rats/group). Rats given naloxone alone did not differ significantly ($p>0.05$) from control rats shown above. V*=$p<0.05$ relative to control group. ‡$p<0.05$ relative to group given 2-chloroadenosine only.

information on binding of adenosine to sites in brain and description of its potent electrophysiological effects suggest that adenosine may play a role in normal brain function [37], perhaps even serving as a transmitter [25]. Binding studies with derivatives of adenosine have been used to characterize possible receptors for adenosine. Specific binding of 2CA to brain synaptosomes, with 2 specific sites visible (KD=1.3 and 10 nM) has been observed [41]. The highest density of receptors was in the caudate nucleus and hippocampus, and binding could be inhibited by PIA (IC_{50}=1.1 nM) and theophylline (IC_{50}=8.8 μM). In bovine brain membrane bound or solibilized A_1 receptors labeled with ^{3}H-N^6-cyclohexyl-adenosine, PIA had an IC_{50} 1/100 that of 2CA and theophylline 1000 × that of 2CA [17]. The doses of ICV theophylline used in the present studies were limited by the solubility of the drug. If the above IC_{50} values reflect receptor affinity, perhaps the limit of only a 20-fold excess of methylxanthine relative to 2CA explains our inability to fully block the respiratory depression produced by 2CA.

The difficulty in assigning biochemically derived receptor site designations to behavioral or physiological responses has previously been noted with adenosine and its derivatives. Adenosine receptor sites have been characterized by the effect of adenosine on adenylate cyclase, A_1 denoting receptors which inhibit and A_2 receptors which stimulate adenylate cyclase activity. Phillis and Wu [37] have recently reviewed the electrophysiological effects of adenosine in CNS slices and concluded that receptors studied in their investigation differed from both A_1 or A_2. Burnstock [5] proposed that purine derivatives may stimulate two types of receptors, P_1 or P_2. One difference between these two receptors was that methylxanthines do not block P_2 receptors. Since aminophylline by one route partially blocked the respiratory effects of 2CA in the present experiments, the respiratory effects would put this receptor as a P_1 type, if Burnstock's classification is used. Eldridge *et al.* [11] and Hedner *et al.* [22] have seen respiratory changes after PIA in the cat and rat, respectively, which closely parallel the present results with 2CA. Both of these workers noted that

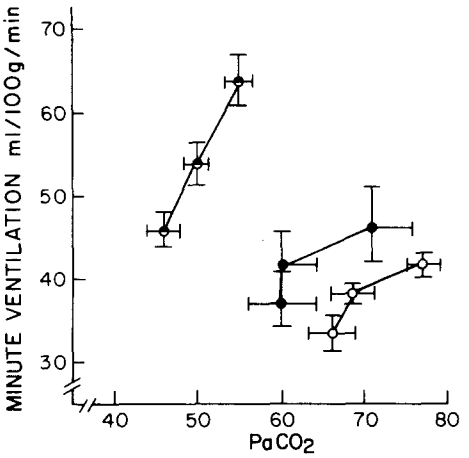

FIG. 9. Antagonism of 2CA-induced changes in the minute ventilation-$PaCO_2$ response curve by naloxone. Each value represents the mean±SEM (brackets) of 5 or 6 rats. Pre-drug values (half open circles) were calculated using values of rats which subsequently received PIA only or naloxone and PIA (see the "Method" section). Post-drug values for rats given PIA only (open circles) or naloxone one minute before PIA (solid circles) were obtained 15–25 minutes after PIA administration (see the "Method" section). Using the least squares method, the slope of the curve for rats given PIA with or without naloxone is significantly less than that of controls ($p<0.01$) but not different from each other. The individual means of the three points in the PIA plus naloxone curve are not significantly different from the corresponding points on the curve of animals given only PIA.

aminophylline antagonized the depression induced by PIA and in the case of the rat, naloxone did not block the depression observed with PIA (Hedner *et al.*, personal communication). The small insignificant ($p>0.05$) antagonism by naloxone of the effect of PIA on the minute ventilation-$PaCO_2$ response curve noted in the present study confirms that observation (Hedner *et al.*, personal communication).

Others have reported that antagonism of responses to adenosine analogues may be specific for different functions altered by these compounds. For example, Dunwiddie and Worth [10] noted that the spectrum of anticonvulsant properties of PIA and 2CA were somewhat different, and not all of the effects of the former were antagonized by aminophylline, where the 2CA anticonvulsant effects were totally reversed.

Neuropharmacology data suggest that the effects of adenosine may be secondary to presynaptic modulation of more classical neurotransmitter systems. Both 2CA and adenosine have been shown to inhibit the release of dopamine from brain slices and synaptosomes [19]. This inhibition was not antagonized by 10^{-4} M theophylline [19,28]. If a similar mechanism were responsible for the 2CA respiratory response, the activity of 2CA should be sharply reduced in animals deficient in dopamine-containing nerve terminals. Our results, however, demonstrated a response to 2CA in rats which received 6OHDA neonatally which was similar to that of control rats. Thus, respiratory depression after 2CA is not exclusively due to effects on dopaminergic neurones.

Earlier results [33] suggested that destruction of serotonergic fibers with neonatal 5,7-DHT enhanced the aminophylline-induced respiratory stimulation when given to adults. Since CNS serotonin neuronal activity appears to reduce respiration, perhaps serotonin-containing neurones might be activated by 2CA, thus depressing respiration. However, the effectiveness of 2CA as a respiratory depressant was not altered in 5,7-DHT pretreated rats, thus the response to 2CA is not solely due to altered serotonergic neurone activity.

What other neurochemical mechanisms could explain the 2CA respiratory response? Murray *et al.* [36] have noted that 83 nM of 2CA did not change acetylcholine or choline content in several areas of brain, yet hippocampal and frontal cortex (but not striatal) turnover of acetylcholine was reduced. This reduction was antagonized by pretreatment with 278 nM theophylline. Similar changes to 2CA were noted with 65 nM PIA. Haubrich *et al.* [20] also observed that 10 μg 2CA ICV decreased the turnover rate of acetylcholine in rat brain hippocampus and cortex but not striatum. Many years ago Miller [30] observed that central administration of cholinergic agonists increased respiration. Thus, 2CA-induced inhibition of cholinergic activation of respiratory centers could explain the adenosine mediated respiratory depression.

The ability of naloxone to at least partly antagonize the respiratory depressant effects of 2CA could be explained by at least three possibilities: (1) the adenosine analogues stimulate opiate receptors; (2) Naloxone combines with and thus blocks purinergic as well as narcotic receptors; and (3) 2CA and PIA produce a release of endogenous opiate peptides, which then would subsequently contribute to the depression of respiration [31]. It seems unlikely that morphine and adenosine share a common receptor which could be blocked by naloxone, since in the myentericplexus-longitudinal muscle preparation of the guinea pig naloxone did not antagonize the effects of adenosine at doses which altered morphine effects on the twitch response [38]. We are not aware of any data on the ability of 2CA or PIA to displace narcotic agonist or antagonist binding to opiate receptors. Although Yarbrough and McGuffin-Clineschmitt [42] have observed that ICV administration of 2CA to mice produced an increase in hot plate reaction times, this effect could be antagonized by theophylline but not by naloxone (10 mg/kg IP 5 minutes before 2CA). Moreover, Williams and Risley [41] failed to observe any antagonism of binding of ^{3}H-2CA to rat brain synaptic membranes by 100 μM naloxone. These data would suggest specific and separate opiate and purinergic receptors, both in the peripheral and central nervous system.

Concerning the third possibility, several workers have proposed the reverse, i.e., that narcotics release endogenous purines. Stone and Perkins [40] proposed that morphine inhibition of the gut was a consequence of adenosine release by the opioid. Fredholm and Vernet [15] observed that morphine application to rat cortical slices induced an increase in release of purines. However, no similar suggestive data is available on endorphin, dynorphin, or enkephalin release after adenosine analogue administration. It is obvious that antagonism of a biological response by naloxone does not necessarily prove that endogenous opiates mediate the response, but is only one requirement [21]. Naloxone has also been reported to have some activity as a GABA antagonist [9]. Horita and Carino [23] have reported that naloxone antagonism of pentobarbital-induced sedation may involve a cholinergic interaction. Thus, the naloxone reversal data of the present study do not necessarily pinpoint a specific neurochemical mechanism, though they do suggest avenues for future work.

Naloxone has been demonstrated to have marked effects on pathological states in animal models. Naloxone has been shown to increase survival in rats subjected to hemorrhage or dogs with endotoxemic shock [12] and in dogs with hypovolemic shock [18]. This beneficial effect is not secondary to the release of catecholamines [14]. Faden *et al.* [13] have observed that naloxone also improved the physiological and neurological recovery from experimental spinal cord injury in the cat perhaps by increasing blood pressure and spinal cord blood flow. Hosobuchi *et al.* [24] observed that 1 mg/kg naloxone could reverse stroke induced by carotid occlusion in gerbils and that similar beneficial effects were seen in man. Cherniack and Craig [6] have reported that newborn rabbits asphyxiated *in utero* had better perinatal scores if the mother had been given naloxone before asphyxiation. The effectiveness of naloxone to block a portion of the effects of 2CA on respiration in the present study raises questions concerning the possible involvement of adenosine in these other models. If 2CA and PIA accurately mimic the effects of endogenous adenosine, then many of the above responses after naloxone may be secondary to antagonism of the effects of adenosine induced by altering the function of opiate mechanisms, perhaps the release of endogenous opiate peptides.

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