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Adenosine deaminase and adenosine uptake inhibitions facilitate ventilation in rats

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Summary. The effects of intracarotid (i.c.) infusions of the adenosine deaminase inhibitor, erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) and of the adenosine uptake blocker, dipyridamole on spontaneous ventilation were studied in rats anaesthetized with sodium pentobarbitone. Both EHNA and dipyridamole mimicked the excitatory effect of adenosine on respiration increasing in a dose-dependent manner respiratory ventilation determined as increases in tidal volume (V_T), respiratory frequency (f) and minute volume ($\dot{V}_E$). These excitatory effects were abolished after section of the carotid sinus nerves. The excitatory effect of EHNA on respiration was prevented by adenosine deaminase and antagonized by 1,3-dipropyl-8(p-sulfophenyl)xanthine (DPSPX). DPSPX also antagonized the excitatory effect of dipyridamole on respiration. Both EHNA and dipyridamole in doses virtually devoid of effect on respiration potentiated the excitatory effect of exogenous adenosine on respiration. Two different effects on respiration were observed during i.c. infusions of cumulative doses of DPSPX: one inhibitory, not present in glomectomized animals and another, excitatory, present in both glomectomized and non-glomectomized animals. It is concluded that endogenous adenosine could be involved in respiration mediated through carotid body chemoreceptors and that the nucleoside is inactivated at this level by deamination and uptake.

Key words: Adenosine – EHNA – Dipyridamole – Respiration – Rat carotid body

Introduction

It has been shown that intracarotid (i.c.) administration of adenosine and its analogues in cats increase carotid body chemosensory activity (McQueen and Ribeiro 1981), and in rats stimulate spontaneous respiration mediated through carotid body chemoreceptors (Monteiro and Ribeiro 1987a). These effects were both antagonized by xanthines and the relative potency of the stable adenosine agonists with 5′-N-ethylcarboxamidoadenosine > 2-chloroadenosine > L-N^6-phenylisopropyladenosine, D-N^6-phenylisopropyladenosine, is compatible with an A_2 subtype of adenosine receptor (McQueen and Ribeiro 1986; Monteiro and Ribeiro 1987a).

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In man, it has also been described that adenosine stimulates spontaneous respiration (Watt and Routledge 1985; Fuller et al. 1987; Biaggioni et al. 1987; Reid et al. 1987), an effect interpreted as a consequence of carotid body chemoreceptor activation (Watt et al. 1987).

A role for endogenous adenosine in the modulation of respiration mediated through the carotid body chemoreceptors could be established if it were found that the adenosine deaminase inhibitor, erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) (Agarwal et al. 1977) and the adenosine uptake blocker dipyridamole (e.g. Stafford 1966) mimic the excitatory effect of adenosine on spontaneous ventilation, as well as if adenosine antagonists decrease the responses to these substances. Excitatory effects of dipyridamole on cat carotid body chemoreceptor activity have been described (McQueen and Ribeiro 1983). Preliminary accounts of parts of the work have been presented (Monteiro and Ribeiro 1987b; Ribeiro et al. 1988).

Methods

Experiments were performed on Wistar rats weighing approximately 400 g, anaesthetized with sodium pentobarbitone (60 mg · kg^{-1} i.p.) supplemented as required during the experiments. The trachea was cannulated and spontaneous ventilation was monitored by a pneumotachygraph (Fleisch no. 0000) connected to a differential transducer (HSE SP 2040D). Respiratory airflow ($\dot{V}$) was obtained by means of a bridge coupler (HSE 570) connected to the differential transducer. Respiratory frequency (f) and tidal volume (V_T) were obtained from a respiratory rate coupler (HSE 568) and from an integrator coupler (HSE 572) respectively, triggered by $\dot{V}$. The respiratory parameters V_T, f and $\dot{V}$ were recorded continuously on a Watanabe WR 3101 Mark VII three channel thermo-oscillographic recorder. Arterial blood pressure (BP) was monitored by means of a catheter introduced into the right femoral artery connected to a Statham P.23 db pressure transducer, and heart rate (HR) was obtained using a cardiotachometer coupler (Sanborn 350-3400 A) triggered by the pressure pulse. BP and HR were recorded continuously on a Hewlett Packard 7700 four channel recorder.

Experimental protocol. Intracarotid (i.c.) injections and/or infusions of drugs were made through a steriflex catheter (Vygon 160-07) introduced via the external carotid artery, with its tip positioned into the common carotid artery just below the bifurcation. Only one drug, or one drug in the

absence or in the presence of another drug were tested per animal. Dose-response curves correspond to the effects obtained with injections or infusions of cumulative doses (usually four) in each rat. Drug injections were made in a volume of 0.1 ml and washed in with 0.2 ml 0.9% w/v aqueous sodium chloride (saline) warmed (37° C) solution, injection time was about 5 s. Drug infusions were made using a Braun perfusion pump at a rate of 0.5 ml · min^{-1} during 1 min except for some adenosine deaminase (EC 3.5.4.4) infusions which were made at a rate of 0.1 ml · min^{-1} during 8 min or 13 min. The intervals between drug injections or infusions were at least 5 min. Exogenous adenosine was administered as control in all experiments since 1) adenosine excites respiration mediated through carotid body chemoreceptors (Monteiro and Ribeiro 1987a) and 2) the drugs tested in the present work interfere with adenosine inactivation (EHNA, dipyridamole and adenosine deaminase) or are adenosine antagonists [1,3-dipropyl-8(p-sulfophenyl)-xanthine, (DPSPX)]. So, in the beginning of each experiment an i.c. injection of 100 nmol of adenosine was given to the animal and to be certain that all adenosine has been washed out from the catheter injections of 0.3 ml saline, usually two to three injections, were administered until no detectable changes occurred in ventilation. Injections and/or infusions of drug vehicles, saline solution, dimethylsulphoxide (DMSO) (in the experiments with dipyridamole) and adenosine deaminase vehicle in the same volume used for drug injections and/or infusions were also administered. Saline administration did not cause detectable modifications either in the respiratory or in the circulatory parameters. DMSO in the maximum concentration (20% v/v) present in the dipyridamole infusions caused a slight increase (16%) in respiratory minute volume ($\dot{V}_E$ – calculated as the product of f and V_T), but no detectable changes in BP or HR were observed. Adenosine deaminase vehicle, a 1/15.4 dilution of a solution containing 50% glycerol and 10 mM KH_2PO_4, caused a $2 \pm 2.2\%$ decrease in $\dot{V}_E$, a $1.7 \pm 2.3\%$ increase in BP and a $0.5 \pm 0.7\%$ decrease in HR ($n = 4$). The adenosine antagonist, DPSPX i.c. was infused either alone or simultaneously with EHNA or with dipyridamole. In the experiments with adenosine deaminase the enzyme was continuously infused i.c. during 8 min [experiments with adenosine or the adenosine analogue, 2-chloroadenosine (CADO)] and during 3 or 13 min [experiments with EHNA or sodium cyanide (NaCN)], and test drugs were injected through the same catheter 3 min before finishing the infusions (except for the 3 min adenosine deaminase infusions, during which EHNA was injected at the 2nd min). The effects of EHNA, NaCN, adenosine and CADO injected i.c. in the presence of adenosine deaminase were compared with those obtained after their i.c. injection during saline or adenosine deaminase vehicle infusions. To know whether the respiratory effects were mediated through carotid body chemoreceptors, drugs were administered before and after bilateral section of the carotid sinus nerves (glomectomized animals). In these experiments i.c. injections of a single dose (200 nmol) of NaCN were given to the animals to verify if chemoreceptor activity was still present. A decrease ($29 \pm 5\%$, $n = 6$) in the basal values for respiratory frequency (f) was consistently obtained after bilateral section of the carotid sinus nerves (see e.g. Fig. 5A). In all animals the vagal nerves were sectioned low in the neck on both sides.

Control values for f, V_T, BP and HR correspond to the mean values measured in a period of 12 s immediately before drug administration. After drug administration the values of f, V_T, BP and HR, were taken as the maximal effects measured during the period of 24 s that followed drug injections or during drug infusions, and were compared with those measured during the control. The maximal effects induced by i.c. injections of the drugs always occurred in the first 24 s that followed the end of the injections. When drugs were infused i.c., the maximal inhibitory or excitatory effects were usually detected in the last 10–20 s of the infusions.

Statistical analysis. Results are expressed as mean values ± SEM. The parameters quantified before and after each test drug administration were compared using the Student's paired t-test. Probability values corresponding to $p < 0.05$ or less were considered statistically significant.

Drugs. Drugs were prepared in 0.9% w/v aqueous sodium chloride solution except for dipyridamole which was initially made up into a 2 mM stock solution in dimethylsulphoxide (DMSO). Doses referred to are those of the salts. The drugs used were: sodium pentobarbitone (Abbott, Chicago, IL, USA), sodium cyanide (NaCN) (Allied Chemical, New York, NY, USA), adenosine, adenosine deaminase type VI (Sigma, St. Louis, MO, USA), dipyridamole (Boehringer, Ingelheim, FRG), 1,3-dipropyl-8(p-sulfophenyl)xanthine (DPSPX) (RBI, Wayland, MA, USA), erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) was a gift or purchased from Burroughs Wellcome Co., Research Triangle Park, NC, USA.

Results

The effect of EHNA

Figure 1 illustrates the effects of i.c. infusion of EHNA (500 nmol · min^{-1}, during 1 min) on $\dot{V}$, V_T, f, HR and BP recorded from a rat where adenosine (100 nmol) was previously injected i.c. As can be seen, adenosine caused its usual excitatory effect on respiration, associated with a slight decrease in BP, and EHNA mimicked this effect of the nucleoside; it increased both V_T and f of the respiratory movements. This effect of EHNA was detected in the first 20 s after starting the infusion was maximal in the next 20 s, and disappeared in 2–3 min after finishing the EHNA infusion (not shown). The excitatory effect of the EHNA infusion on respiration was associated with a small decrease in BP (Fig. 1) without marked changes in HR.

EHNA (50–1000 nmol · min^{-1}, during 1 min, $n = 4$–6) increased in a dose-dependent manner, respiratory ventilation determined as increases in $\dot{V}_E$ (see Fig. 6). The ED_{25}, determined as the dose of EHNA that increased $\dot{V}_E$ by 25% was 199 nmol. Small but statistically significant ($p < 0.05$) decreases ($12 \pm 2\%$, $16 \pm 2\%$ and $20 \pm 5\%$) in BP were observed in these experiments after infusions of high doses (300–1000 nmol · min^{-1}, during 1 min) of EHNA. No detectable changes in HR were observed after i.c. infusions of EHNA.

After bilateral section of the carotid sinus nerves both the effects of the chemoreceptor stimulant, NaCN (200 nmol), and of EHNA (500 nmol) on respiration disappeared (data not shown).

Adenosine deaminase alone (10 IU in 0.1 ml · min^{-1}, during 13 min or 50 IU in 0.5 ml · min^{-1}, during 3 min) did

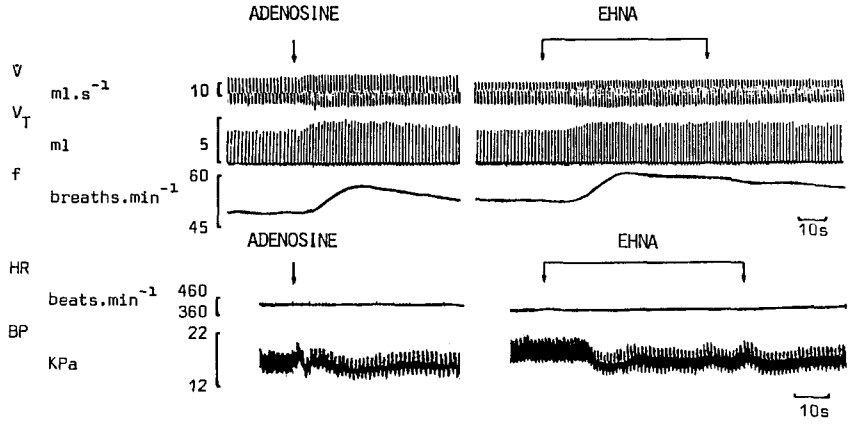

Fig. 1. Comparison of the effects of erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) (500 nmol · min^{-1}, during 1 min) with those of adenosine (100 nmol, i.c.) on airflow ($\dot{V}$), tidal volume (V_T), respiratory frequency (f), heart rate (HR) and arterial blood pressure (BP) of a rat anaesthetized with pentobarbitone, vagotomized and with the carotid sinus nerves intact. EHNA was infused approximately 60 min after the injection of adenosine, and during this period a supplement of pentobarbitone (10% of the initial dose) was administered about 15 min after the injection of adenosine. The effect of EHNA shown, is the result of the fourth infusion of a series of four cumulative and consecutive infusions of 50, 100, 300 and 500 nmol · min^{-1}, during 1 min respectively, given with intervals of at least 5 min

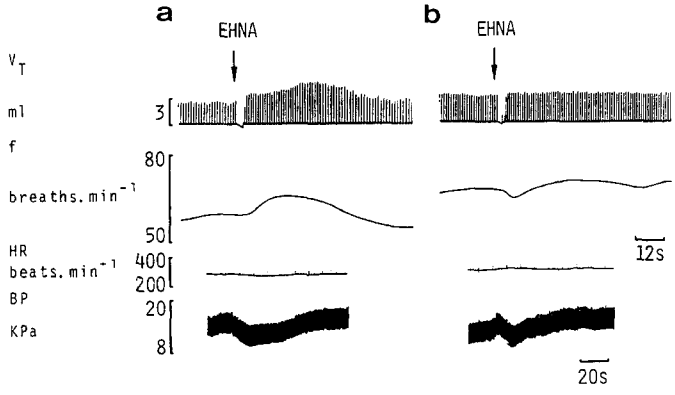

Fig. 2. Effects of i.c. injections of erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) (500 nmol) on tidal volume (V_T), respiratory frequency (f), heart rate (HR) and arterial blood pressure (BP) of a rat. (**a**) During i.c. infusion of adenosine deaminase vehicle (0.5 ml · min^{-1}, during 3 min). (**b**) During adenosine deaminase i.c. infusion (50 IU in 0.5 ml · min^{-1}, during 3 min). EHNA was injected at the 2nd min after starting adenosine deaminase vehicle (**a**) or adenosine deaminase (**b**) infusions. The very transient inhibitory effect on respiration observed in this animal during the time (5 s) that lasted the EHNA injections was also observed during saline injections without being followed by excitatory effects on respiration. Note that (f) shown in (**b**) before administering EHNA in the presence of adenosine deaminase, was of the same order as that recorded during the 3 min that preceeded the beginning of the adenosine deaminase infusion (not shown in the figure)

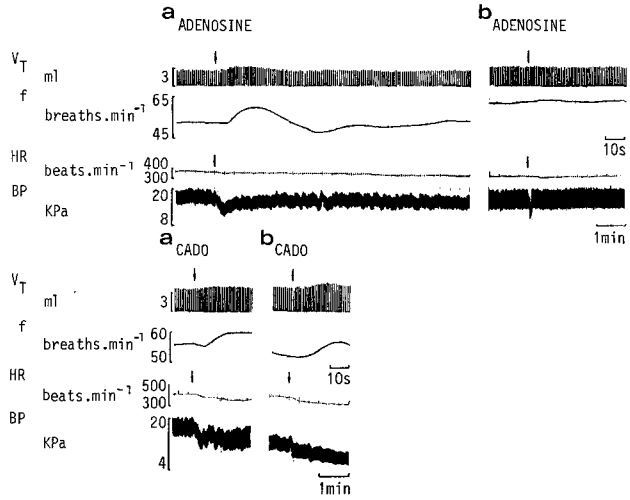

Fig. 3. Effects of i.c. injections of adenosine (10 nmol) and of 2-chloroadenosine (CADO) (10 nmol) on tidal volume (V_T), respiratory frequency (f), heart rate (HR) and arterial blood pressure (BP) of a rat. (**a**) During saline infusion (0.1 ml · min^{-1}, during 8 min, i.c.). (**b**) During adenosine deaminase infusion (10 IU in 0.1 ml · min^{-1}, i.c., during 8 min). Drugs were injected at the 5th min after starting saline (**a**) or adenosine deaminase (**b**) infusion

not significantly ($p > 0.05$, $n = 5$) modify $\dot{V}_E$, HR or BP ($3.8 \pm 4.8\%$ increase for $\dot{V}_E$, $4.5 \pm 1.4\%$ increase for BP and $0.4 \pm 0.7\%$ decrease for HR), but prevented the respiratory stimulation caused by EHNA (500 nmol), and decreased the duration of the hypotensive effect induced by EHNA (see Fig. 2). These effects should not be attributed to the adenosine deaminase vehicle, since both the excitatory effect of EHNA (500 nmol) on $\dot{V}_E$ and its hypotensive effect were present during infusion of the adenosine deaminase vehicle (0.5 ml · min^{-1}, during 3 min) (see Fig. 2a). As shown in Fig. 3 adenosine deaminase (10 IU in 0.1 ml · min^{-1}, during 8 min) prevented the excitatory effect of adenosine (10 nmol, i.c.) on respiration. The transient fall in BP after the i.c. injection of adenosine (Fig. 3) still remained after its administration in the presence of adenosine deaminase, though it became shorter (Fig. 3). The excitatory effect of the adenosine stable analogue 2-chloroadenosine (CADO) (10 nmol, i.c.) on respiration and its hypotensive effect were not prevented when CADO was given during the adenosine deaminase infusion (10 IU in 0.1 ml · min^{-1}, during 8 min^{-1}) (Fig. 3). To preclude that the prevention of the excitatory effect of EHNA on respiration by adenosine deaminase would result from inactivation of carotid body chemoreceptors by this enzyme, injections of NaCN were performed before and during the adenosine deaminase infusion. Adenosine deaminase (10 IU in 0.1 ml · min^{-1}, during 13 min) was not able to prevent the excitatory effect on

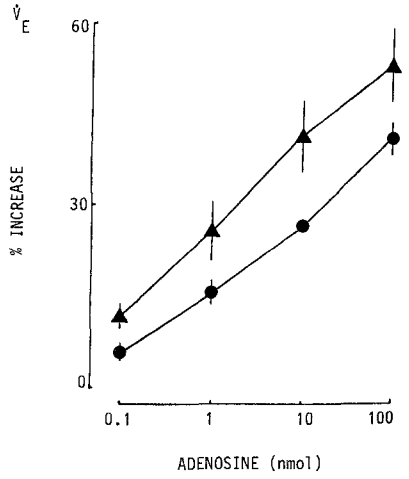

Fig. 4. Effects of adenosine on respiratory minute volume ($\dot{V}_E$) before (●) and in the presence (▲) or erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) (10 nmol). Doses of adenosine are plotted on a log 10 scale and the effect on $\dot{V}_E$ is expressed as % increase in ml · min^{-1} observed in the first 12 s that followed the end of the injections. 0% represents absolute values determined for the 12 s that preceded the injections. Absolute values before the injection of adenosine in the absence and in the presence of EHNA were 146 ± 33 ml · min^{-1} and 160 ± 65 ml · min^{-1} respectively. Each *point* is the average of 3 experiments and *vertical bars* represent ± SEM and are shown when they exceed the symbols

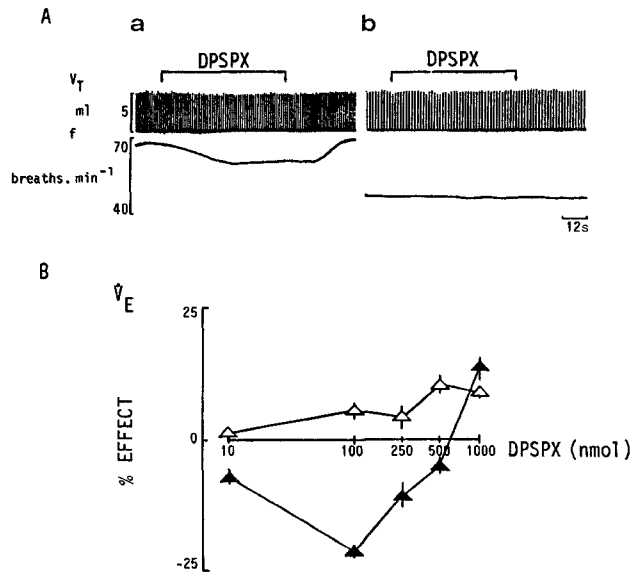

Fig. 5. Effects of 1,3-dipropyl-8-(p-sulfophenyl)xanthine (DPSPX) i.c. infusion, on spontaneous ventilation of rats. (**A**) Effect of DPSPX 100 nmol on tidal volume (V_T) and respiratory frequency (f) of a rat with intact carotid sinus nerves (**a**) and a rat after bilateral section of the carotid sinus nerves (**b**). (**B**) Dose-response curves for the effect of cumulative infusions of DPSPX, given with intervals of at least 5 min, on respiratory minute volume ($\dot{V}_E$) of rats with intact carotid sinus nerves (▲) and after bilateral section of the carotid sinus nerves (△). Doses are plotted on a log 10 scale and the effects expressed as % increase or decrease in ml · min^{-1} quantified in the last 12 s of the infusions. 0% represents the absolute values ± SEM determined for the 12 s that preceded the infusions and were 268 ± 15 ml · min^{-1} for ▲ and 193 ± 14 ml · min^{-1} for △. Each *point* is the average of 3 – 5 experiments for ▲ and of 2 experiments for △. *Vertical bars* represent ± SEM

respiration caused by NaCN (200 nmol, i.c.) injections (data not shown).

Adenosine. Adenosine infusions (100 nmol · min^{-1}, i.c., n = 5) were also performed during the same period (1 min) as that used for EHNA (500 nmol · min^{-1}, i.c.) infusions. In these conditions adenosine increased respiratory ventilation ($\dot{V}_E$) (data not shown), an effect which was detected about 23 ± 4 s after starting the infusions, was maximal (64 ± 8% increase in $\dot{V}_E$) in the next 16 ± 5 s, and remained at this level during the time of infusion. $\dot{V}_E$ returned to the control level in about 1 – 2 min after stopping the infusion. The effects of i.c. injections of adenosine on $\dot{V}_E$ were also investigated, and the dose-response curves obtained in the absence and in the presence of EHNA are shown in Fig. 4. EHNA was injected i.c. in a dose (10 nmol) which alone did not cause detectable modifications in respiration, BP or HR, but when injected simultaneously with adenosine shifted the dose-response curve for adenosine to the left by a factor of approximately 10 (see Fig. 4), indicating that EHNA potentiates the excitatory effect of exogenous adenosine on respiration. The hypotensive effect induced by adenosine was also more pronounced (2.6 and 2.2 times for the doses of 10 and 100 nmol respectively) in the presence of EHNA (10 nmol, n = 3) (data not shown). EHNA used in the dose (10 nmol) that potentiated the effect of adenosine, did not increase the excitatory effect of the adenosine stable analogue, CADO (0.01 – 10 nmol i.c., n = 3), on respiration (data not shown).

Alkylxanthine. It was previously described (Monteiro and Ribeiro 1987a) that theophylline antagonizes the excitatory effect of i.c. injections of CADO on $\dot{V}_E$. As the excitatory effect of EHNA on ventilation could be attributed to increasing the levels of endogenous adenosine we performed experiments with EHNA and the adenosine antagonist, 1,3-dipropyl-8(p-sulfophenyl)xanthine (DPSPX). DPSPX was used since it is a potent water-soluble (more potent than theophylline in terms of adenosine antagonism) antagonist for adenosine receptors (Daly et al. 1985).

Cumulative infusions i.c. of the potent adenosine antagonist DPSPX (100 and 250 nmol · min^{-1}, during 1 min) in animals (n = 3 – 5) with intact carotid sinus nerves, caused small but consistent reductions in $\dot{V}_E$. These reductions were maximal for the dose of 100 nmol (see Fig. 5). In the doses of 10 and 500 nmol, DPSPX caused little or no effect on respiration, and an increase in $\dot{V}_E$ was obtained after the infusion of 1 μmol. In two animals in which the carotid sinus nerves have been previously cut (glomectomized animals), i.c. infusions of DPSPX (100 and 250 nmol · min^{-1}, during 1 min) did not cause its usual inhibitory effect on V_T and f (Fig. 5), and the virtual absence of effect of 500 nmol of DPSPX was changed into a small excitatory effect. A slight, non-significant and apparently not dose-dependent increase in BP (4.7 ± 1.2% for non-glomectomized animals, n = 3 – 5, and 5.7 ± 1% for glomectomized animals, n = 2) was observed during DPSPX infusions (10 – 1000 nmol); in the same conditions no detectable changes in HR were observed.

Antagonism of the effect of EHNA by DPSPX, was tested by using the two doses (10 and 500 nmol · min^{-1}) of DPSPX, which on its own were almost devoid of effect on respiration. With the lower dose (n = 5) the excitatory effect of EHNA on respiration was not consistently modified (data

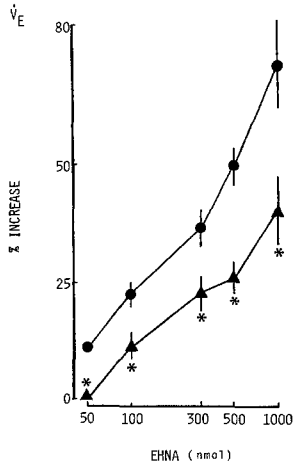

Fig. 6. Dose-response curves for the effects of i.c. infusions of erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) (50–1000 nmol · min^{-1}, during 1 min) on respiratory minute volume ($\dot{V}_E$) before (●) and in the presence (▲) of 1,3-dipropyl-8-(p-sulfophenyl)xanthine (DPSPX) (500 nmol · min^{-1}, i.c., during 1 min). Doses of EHNA are plotted on a log 10 scale and the effect on $\dot{V}_E$ is expressed as % increase in ml · min^{-1} observed in the last 12 s of the infusions. 0% represents absolute values determined for the 12 s that preceded the infusions. Absolute values for $\dot{V}_E$ immediately before the infusions of EHNA in the absence and in the presence of DPSPX were 147 ± 3 ml · min^{-1} and 160 ± 5 ml · min^{-1}, respectively. Each *point* is the average of 4–6 experiments and *vertical bars* represent ± SEM. * $p < 0.05$ (Student's paired *t*-test related to the values, expressed as % increase, in the absence and in the presence of DPSPX

not shown), but as illustrated in Fig. 6, DPSPX (500 nmol · min^{-1}, during 1 min, $n = 4-6$) shifted to the right the dose-response curve obtained for EHNA (50–1000 nmol · min^{-1}, during 1 min) on $\dot{V}_E$ by a factor of approximately 3. Antagonism (approximately 50%, $n = 4-6$) of the hypotensive effect induced by EHNA (300–1000 nmol) was also observed when EHNA was infused in the presence of DPSPX (500 nmol) (data not shown).

The effect of dipyridamole

Figure 7A illustrates the effects of i.c. infusion of dipyridamole (200 nmol · min^{-1}, during 1 min) on $\dot{V}$, V_T and BP recorded from the same animal where NaCN has been previously administered (see Fig. 7A). Both NaCN (200 nmol) and dipyridamole (200 nmol · min^{-1}) were administered before (Fig. 7Aa) and after (Fig. 7Ab) bilateral section of the carotid sinus nerves. When dipyridamole was infused before cutting the sinus nerves it caused an increase in the respiratory parameters (V_T and $\dot{V}$). The excitatory effect of dipyridamole on ventilation could be detected in the first 30 s that followed the beginning of its i.c. infusion, was maximal in the last 10 s of the infusion and usually, depending on the doses, lasted approximately 5 min after the end of the infusions. The excitatory effect of the dipyridamole infusion was associated with a small decrease (about 10%) in BP (see Fig. 7A). After bilateral section of the carotid sinus nerves (Fig. 7Ab) both the excitatory effect of dipyridamole and of NaCN on the respiratory parameters disappeared, but the decrease in BP remained. In Fig. 7B are quantified the effects of dipyridamole i.c. infusions (20–200 nmol · min^{-1}, during 1 min) on $\dot{V}_E$, BP and HR, and is shown that both the excitatory effect of dipyridamole on ventilation and its inhibitory action on BP were dose-dependent. The ED_{25}, for the excitatory effect of dipyridamole on respiration, determined as the dose of dipyridamole that increased $\dot{V}_E$ by 25% was 79 nmol. Small but statistically significant ($p < 0.05$) decreases in BP were observed with infusion of high doses (150–200 nmol) of dipyridamole (Fig. 7B).

Adenosine. Dose-response curves for the excitatory effects of i.c. injections of adenosine on $\dot{V}_E$ obtained in the absence and in the presence of dipyridamole are shown in Fig. 8. Dipyridamole was injected i.c. in a dose (20 nmol), which alone did not cause detectable modifications in respiration, BP or HR, but when injected simultaneously with adenosine shifted the dose-response curve for adenosine to the left (see Fig. 8), indicating that dipyridamole potentiates the excitatory effect of exogenous adenosine on respiration without apparently potentiating the hypotensive effect induced by exogenous adenosine. Administered in the same dose (20 nmol, i.c., $n = 2$) dipyridamole did not potentiate the excitatory effect on respiration caused by i.c. injections of the stable adenosine analogue, CADO (0.01–10 nmol) (data not shown).

Alkylxanthine. To know if the excitatory effect of dipyridamole was mediated by increasing the levels of endogenous adenosine, we performed experiments in which dipyridamole was infused i.c. in the absence and in the presence of DPSPX. Figure 9 shows dose-response curves for the excitatory effects of dipyridamole (50–200 nmol · min^{-1}, during 1 min) on $\dot{V}_E$ recorded before and in the presence of DPSPX (500 nmol · min^{-1}, during 1 min). In the presence of DPSPX a shift to the right, by a factor of approximately 2, on the dose-response curve of dipyridamole, was obtained (Fig. 9).

Discussion

The present results show that both the adenosine deaminase inhibitor, EHNA and the adenosine uptake blocker, dipyridamole mimic the excitatory effect of adenosine on respiration. These effects of EHNA and dipyridamole were mediated through carotid body chemoreceptors, since these actions disappeared after bilateral section of the carotid sinus nerves.

The excitation caused by EHNA or dipyridamole on ventilation was obtained in rats with vagal nerves cut, precluding that the excitatory effects of EHNA and dipyridamole on respiration depend on vagal integrity (cf. Runold et al. 1987). Furthermore, as previously described in relation to adenosine no marked differences were found between rats with vagal nerves intact or cut (Monteiro and Ribeiro 1987a).

The effect of EHNA appears to be specific, i.e., to be related to its capacity to inhibit adenosine deaminase (Agarwal et al. 1977), since (1) it was prevented by exogenous adenosine deaminase, an enzyme which did not prevent the excitatory effect on respiration of the stable adenosine analogue, CADO, which is not substrate for the enzyme, and (2) EHNA enhanced the excitatory effect of i.c. administration of adenosine, but did not potentiate the excitatory effect caused by CADO. Potentiation of the central actions of

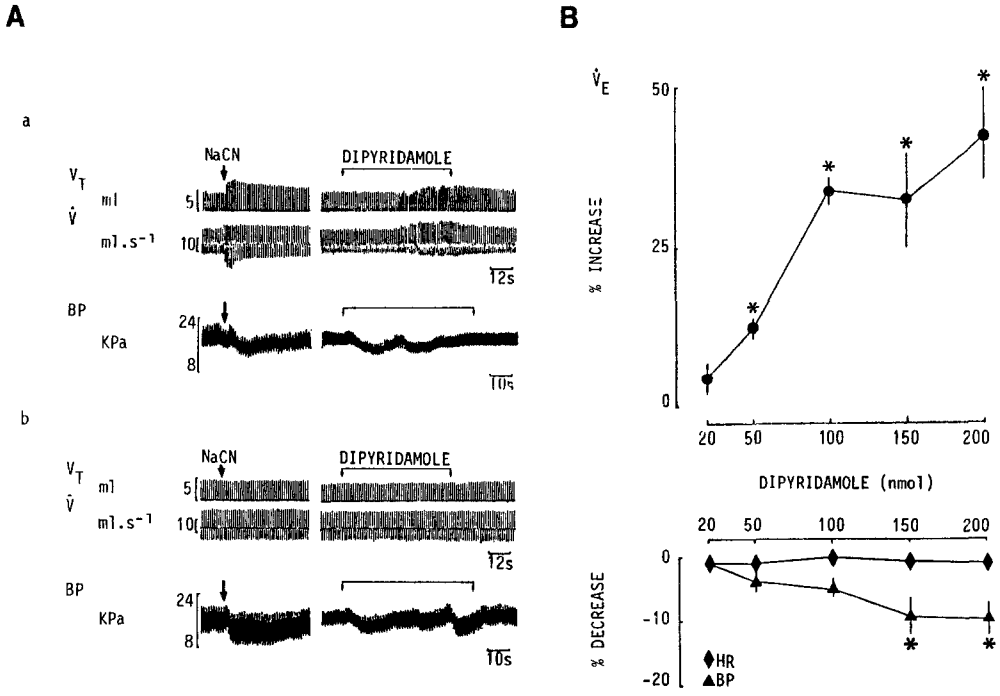

Fig. 7. Effects of intracarotid infusions of dipyridamole. (**A**) Comparison between the effects of dipyridamole (200 nmol · min^{-1}, during 1 min) and of injections of NaCN (200 nmol, i.c.) on tidal volume (V_T), airflow ($\dot{V}$) and arterial blood pressure (BP) of a rat before (**a**) and after (**b**) bilateral section of the carotid sinus nerves. (**B**) Dose-response curves for the effects of dipyridamole (20–200 nmol · min^{-1}, during 1 min) on respiratory minute volume ($\dot{V}_E$), arterial blood pressure (BP) and heart rate (HR). Doses are plotted on a log 10 scale and the effects expressed as percentage increase in ml · min^{-1} for $\dot{V}_E$ and percentage decrease in KPa for BP and beats · min^{-1} for HR quantified in the last 12 s of the infusion. 0% represents the absolute values determined for the 12 s that preceded the infusions. Absolute values for $\dot{V}_E$, BP and HR immediately before the infusions of dipyridamole were 131 ± 70 ml · min^{-1}, 15 ± 3 KPa and 412 ± 49 beats · min^{-1} respectively. Each *point* is the average of 5 experiments and *vertical bars* represent $\pm$ SEM. * $p < 0.05$ (Student's paired *t*-test related to the absolute values before and during dipyridamole infusions)

exogenous adenosine by EHNA (Skolnick et al. 1978; Phillis et al. 1985), or increases in the levels of endogenous adenosine by EHNA have been demonstrated in rats (Zetterström et al. 1982); EHNA mimics the decrease in coronary vascular resistance induced by adenosine as well as increases the levels of endogenous adenosine (Heller and Mohrmann 1987). In the present work EHNA also mimicked the inhibitory effect of adenosine on BP which is consistent with the effect of EHNA mediated through increase of endogenous adenosine. EHNA might increase the levels of adenosine at the receptor operating stimulation of respiration through inhibition of: (a) adenosine deaminase located on the cell surface (Andy and Kornfeld 1982); (b) adenosine uptake (Newby 1981; Henderson 1983); (c) intracellular adenosine deaminase with increase in adenosine release (e.g. Heller and Mohrmann 1987; Zetterström et al. 1982); (d) adenylate deaminase with increase in AMP and adenosine (Henderson 1983). However, from the present work it is not possible to know the relative contribution of each of these mechanisms.

Adenosine deaminase administered alone did not modify respiration. This is not an unexpected finding considering that the use of intra-arterial administration of adenosine deaminase as an investigative tool in vivo has limitations (see e.g. Olsson 1987; Rubio et al. 1987) such as (1) low enzyme activity and large deamination reserve at physiological concentrations; (2) high molecular weight and therefore difficulties to penetrate and to achieve homogeneous distribution in the interstitial space; (3) increased production of adenosine with adenosine deaminase infusion and (4) changes in basal circulatory flow during adenosine deaminase infusion modifying the dilution of the enzyme and the washout from the interstitial space. However, in the present work adenosine deaminase prevented the effects of exogenously applied adenosine and of EHNA on respiration, without changing the excitatory effects of substances that are not substrates for the action of the enzyme, such as NaCN or CADO. This suggests that these effects of adenosine deaminase are related to adenosine inactivation.

The effect of EHNA after starting its administration appeared as rapid as that of adenosine itself and remained about the same period after stopping EHNA infusion. These similarities, in the time courses of the effects of adenosine and EHNA, suggest that adenosine deaminase inhibition by EHNA could occur very quickly. This apparently agrees with the results obtained in mice in vivo, where adenosine deaminase inhibition by EHNA appeared to be effective immediately upon injection (Plunkett et al. 1979). Also in vitro where adding adenosine deaminase to a mixture containing EHNA and adenosine, inhibition of adenosine deamination is apparent in the first minute (Agarwal et al. 1977). The finding that EHNA has a $T_{1/2}$ for dissociation of the enzyme-inhibitor complex of about 2.5 min (Agarwal et al. 1977) could explain how the effect of EHNA is so transient. However, one cannot totally preclude that EHNA could have other effects on carotid body chemoreceptors, which might also mimic the adenosine action on respiration. Knowing that the adenosine receptor that mediates the exci-

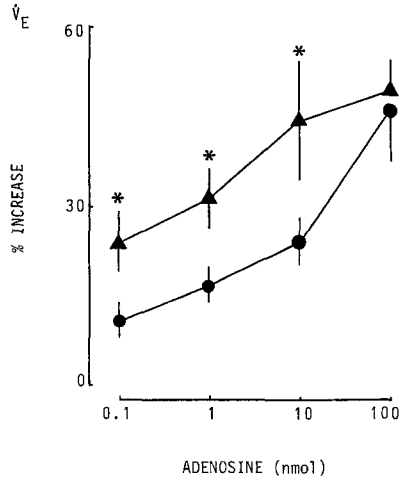

Fig. 8. Effects of adenosine on respiratory minute volume ($\dot{V}_E$) before (●) and in the presence (▲) of dipyridamole (20 nmol). Doses of adenosine are plotted on a log 10 scale and the effect on $\dot{V}_E$ is expressed as % increase in ml · min^{-1} observed in the first 12 s that followed the end of the injections. 0% represents absolute values determined for the 12 s that preceded the injections. Absolute values before the injection of adenosine in the absence and in the presence of dipyridamole were 230 ± 11 ml · min^{-1} and 250 ± 13 ml · min^{-1}, respectively. Each *point* is the average of 4 experiments and *vertical bars* represent ± SEM. * $p < 0.05$ (Student's paired *t*-test related to the values, expressed as % increase, in the absence and in the presence of dipyridamole)

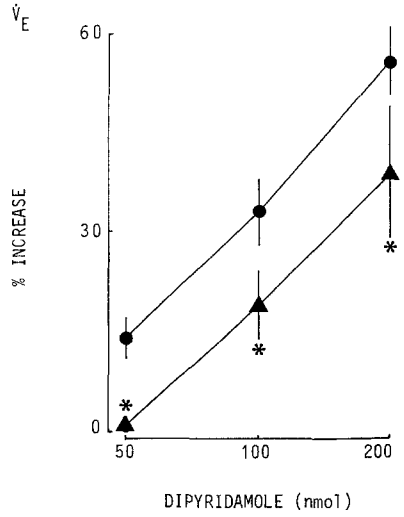

Fig. 9. Dose-response curves for the effects of i.c. infusions of dipyridamole (50–200 nmol · min^{-1}, during 1 min) on respiratory minute volume ($\dot{V}_E$) before (●) and in the presence (▲) of 1,3-dipropyl-8-(p-sulfophenyl)xanthine (DPSPX) (500 nmol · min^{-1}, i.c., during 1 min). Doses of dipyridamole are plotted on a log 10 scale and the effect on $\dot{V}_E$ is expressed as % increase in ml · min^{-1} observed in the last 12 s of the infusions. 0% represents absolute values determined for the 12 s that preceded the infusions. Absolute values for $\dot{V}_E$ immediately before the infusion of dipyridamole in the absence and in the presence of DPSPX were 192 ± 39 ml · min^{-1} and 175 ± 40 ml · min^{-1}, respectively. Each *point* is the average of 5 experiments and *vertical bars* represent ± SEM. * $p < 0.05$ (Student's paired *t*-test related to the values, expressed as % increase, in the absence and in the presence of DPSPX)

tatory effect of adenosine on respiration is an A_2-adenosine receptor subtype (Monteiro and Ribeiro 1987a) and that dibutyryl cyclic AMP increases carotid body chemosensory activity (Ribeiro and McQueen 1983), one might speculate that if EHNA inhibits phosphodiesterases (Wolberg et al. 1981) it could increase respiration through this mechanism.

Dipyridamole potentiated the excitatory effect of exogenous adenosine but did not potentiate a similar effect on respiration caused by CADO, which has a low affinity for the adenosine transport system (Jarvis et al. 1985). This suggests that dipyridamole excites respiration by inhibiting adenosine uptake (Stafford 1966). An increase in spontaneous chemoreceptor discharge and a potentiation of the excitatory effect of exogenous adenosine on chemoreceptor activity induced by i.c. infusions of dipyridamole in cats have also been described (McQueen and Ribeiro 1983). The finding that in the present work dipyridamole decreased BP, mimicking the effect of adenosine, is consistent with results obtained in humans where dipyridamole, which elevates endogenous levels of adenosine in venous plasma, is used to reduce the dose requirement of adenosine to induce hypotension (Sollevi et al. 1984a; Sollevi et al. 1984b). The excitatory effects observed in the present work with dipyridamole were obtained with infusions of 50–200 nmol/min, which for rats with about 25 ml of blood and assuming an uniform distribution might represent at the end of the infusions concentrations between 2–8 nmol/ml blood. These concentrations are of the same order as the amount of dipyridamole (1 nmol/ml blood) that in human blood causes 90% inhibition of adenosine inactivation (cf. Klabunde 1983). Other mechanisms have been postulated to explain the actions of dipyridamole: phosphodiesterase inhibition (Kukovetz and Pöch 1970), membrane-bound adenosine kinase inhibition (Hopkins and Goldie 1971) and adenosine deaminase inhibition (Deuticke and Gerlach 1966). None of these mechanisms were investigated in the present work, and therefore, one cannot preclude that these mechanisms could contribute to the excitatory effects of dipyridamole on respiratory ventilation, however, it has been demonstrated (Schrader et al. 1972) that dipyridamole in a micromolar (8.5 μM) concentration significantly inhibits the uptake of adenosine in intact red cell ghosts, and in solubilized ghost cells dipyridamole even in higher concentration (100 μM) does not inhibit adenosine kinase or adenosine deaminase.

It was previously described that in humans (Maxwell et al. 1987) theophylline modifies the excitatory effect of adenosine on ventilation. An antagonism by this xanthine was detected in cats (McQueen and Ribeiro 1986) related to the excitatory effect of the adenosine analogue 5′-N-ethylcarboxamidoadenosine on chemoreceptor activity, as well as in rats (Monteiro and Ribeiro 1987a), where theophylline antagonizes the excitatory action of CADO on spontaneous ventilation. In the present work it was used DPSPX, a xanthine which in rats antagonizes the cardiovascular effects of adenosine (Robertson et al. 1988). This xanthine in a concentration virtually devoid of effect on respiration, antagonized the excitatory effects of EHNA and dipyridamole on respiration. These results support that the excitatory effects of both EHNA and dipyridamole on respiration involve the presence of endogenous adenosine. A similar interpretation has been advanced to explain the antagonism by theophylline of the cardiovascular responses to dipyridamole in man (Sollevi 1986) as well as the decrease caused by theophylline on the effects of dipyridamole + EHNA on the vascular responses to electrical stimulation (Sollevi and Fredholm 1983).

In the doses of 100 and 250 nmol, DPSPX infused i.c. caused a consistent inhibitory effect on respiration. This effect appears to be mediated by the carotid body chemoreceptors since it was not observed in the glomectomized animals. In the dose of 500 nmol, which has been used to test the antagonism, DPSPX caused little or no effect on respiration, and in a higher dose (1 μmol) DPSPX increased respiration. This effect probably has in part a central origin since (1) it remained in glomectomized animals (present work), and (2) excitatory effects of high doses (37.7 μmol $\cdot$ kg^{-1}, i.v.) of theophylline on respiration mediated at the level of the brainstem have been demonstrated in cats (Eldridge et al. 1983).

In normoxic and normocapnic cats the adenosine antagonist, theophylline inhibits carotid body chemoreceptor activity (McQueen and Ribeiro 1981), and 8-phenyltheophylline, an adenosine antagonist more potent than theophylline and almost devoid of phosphodiesterase activity (see Smellie et al. 1979) antagonizes the chemoreceptor response induced by hypoxic stimuli (McQueen and Ribeiro 1986). This is of interest since, as previously described (e.g. Berne 1980; Sollevi 1986), hypoxia is the main stimulus to produce release of adenosine in blood, and constitutes the physiological stimulus for carotid body chemoreceptor activity (e.g. Biscoe 1971). The finding that in humans adenosine increases the hypoxic ventilatory response but does not alter the hypercapnic response provides further evidence for the hypothesis that adenosine may have a role in the mechanism of peripheral chemoreception (Maxwell et al. 1986). The results now presented obtained with EHNA, dipyridamole and DPSPX strongly support that endogenous adenosine can exert an excitatory "tone" over respiration mediated through carotid body chemoreceptors.

The present results also indicate how endogenous adenosine could be inactivated suggesting that both deamination and uptake could be involved in the process, as it has been demonstrated either with studies in vivo in rats at the cerebral blood vessels (Phillis et al. 1985) or with studies in vitro, using the rat diaphragm neuromuscular junction, where adenosine seems to be also inactivated through deamination and uptake (Sebastião and Ribeiro 1988).

In conclusion, the findings that adenosine deaminase and adenosine uptake inhibitions facilitate respiratory ventilation mediated through carotid body chemoreceptors, are highly suggestive that endogenous adenosine could be involved in respiration mediated through carotid body chemoreceptors and that the nucleoside is inactivated by deamination and uptake.

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