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Pharmacological characterization of the receptor involved in chemoexcitation induced by adenosine

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1 Experiments were performed on cats anaesthetized with pentobarbitone in which carotid body chemoreceptor activity was recorded from the peripheral end of a sectioned carotid nerve.

2 Intracarotid (i.c.) injections of adenosine and its analogues, NECA (5'-N-ethylcarboxamidoadenosine), L-PIA (L-N^6-phenylisopropyladenosine), and D-PIA (D-N^6-phenylisopropyladenosine), caused dose-related increases in chemosensory discharge. The rank order of potency as chemoreceptor stimulants was: NECA > adenosine > L-PIA > D-PIA.

3 Infusion of theophylline antagonized the chemoexcitatory effects of NECA, and infusion of 8-phenyltheophylline (8-PT), which is a more potent adenosine antagonist with less activity as a phosphodiesterase inhibitor, reduced the chemoexcitation induced by adenosine.

4 Infusion of 8-PT ($10\,\mu g\,min^{-1}$ i.c.), a dose which substantially reduced the effect of injected adenosine, also reduced the sensitivity of carotid chemoreceptors to hypoxia (10% O_2 for 4 min).

5 It is concluded that the adenosine receptors in the cat carotid body which mediate chemosensory excitation are xanthine-sensitive and appear to be of the A_2 sub-type. Adenosine, released within the carotid body by physiological stimuli, may be involved in chemoexcitation.

Introduction

McQueen & Ribeiro (1983) reported that adenosine stimulates carotid body chemoreceptors through an R-type of adenosine receptor (Londos & Wolff, 1977). The R-type adenosine receptor classification encompasses at least two subtypes of receptor, namely A_1 and A_2, which have different pharmacological profiles for agonists (Van Calker *et al.*, 1979). At A_1-adenosine receptors the N^6-substituted adenosine analogue, L-N^6-phenylisopropyladenosine (L-PIA) is more potent than adenosine or 2-chloroadenosine, which in turn are more potent than 5'-N-ethylcarboxamidoadenosine (NECA). At A_2-adenosine receptors the order of agonist potency is reversed, i.e. NECA is more potent than adenosine or 2-chloroadenosine, and these substances are more potent than L-PIA. The present work was undertaken to provide further pharmacological evidence concerning the type(s) of adenosine receptor involved in chemoexcitation. We also wanted to re-examine the effects of adenosine receptor antagonists because in a previous study (McQueen & Ribeiro, 1981) we were unable to demonstrate any antagonism of the chemoexcitatory effect of adenosine by the xanthine theophylline, which is an adenosine (A_1 and A_2) receptor antagonist.

Since hypoxia can induce the release of considerable amounts of adenosine in various tissues (e.g. Berne, 1980), we took the opportunity of investigating the effects of the adenosine antagonist 8-phenyltheophylline (8-PT) (Wu *et al.*, 1982) on the responsiveness of carotid body chemoreceptors to stimulation by hypoxia.

A preliminary account of some of this work has been presented (Ribeiro & McQueen, 1985).

Methods

Experiments were performed on cats anaesthetized with pentobarbitone sodium ($42\,mg\,kg^{-1}$ i.p.), supplemented as required during the experiments, artificially ventilated with air and paralysed with gallamine ($3\,mg\,kg^{-1}$ i.v.). Full details of the experimental procedures have been given previously (McQueen, 1977) and only a brief description follows.

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The lingual and superior thyroid arteries ipsilateral to the carotid nerve from which recordings were obtained were both cannulated, the catheter tips being positioned in the common carotid artery; blood pressure was recorded from a cannulated femoral artery. Electrical activity of chemoreceptor units was recorded from filaments of the peripheral end of a sectioned carotid (sinus) nerve, stored on FM tape, passed through a pulse height (window) voltage discriminator and quantified with the aid of a microcomputer (Commodore 3032). In the majority of the experiments the ganglioglomerular (sympathetic) nerves were cut.

Drugs were dissolved in either modified Locke solution (McQueen, 1977) or 0.9% w/v aqueous NaCl solution, except for 8-phenyltheophylline (8-PT, 500 $\mu g\,ml^{-1}$), which was dissolved in saline containing 2% methanol and 2% M NaOH and diluted with saline to 100 $\mu g\,ml^{-1}$ 8-PT for infusion, and D- and L-PIA which were dissolved in DMSO and then diluted with saline; the strongest solution of PIA used for injection contained 4% DMSO, and 4% DMSO in saline was used as a control for the injections. Drug injections were made in a volume of 0.1 ml into the lingual catheter (i.c.) and washed in with 0.2 ml Locke solution which had been bubbled with 5% CO_2: 95% air in a water bath at 37°C; they were made over a 2 s period. Drug infusions were made into the common carotid artery via the thyroid catheter at a rate of 0.1 ml min^{-1} using a Unita pump (Braun); the catheter dead space was 0.2 ml.

Comparison of the drug effects

The averaged discharge recorded during a post-injection period was computed and expressed as a percentage change from the pre-injection frequency. The ED_{50} (dose causing a 50% increase in discharge during the period) was calculated for adenosine and adenosine receptor agonists before and, in some of the experiments, after administering an adenosine receptor antagonist.

Drugs

The drugs used were: sodium pentobarbitone, gallamine triethiodide (May & Baker); adenosine, theophylline, 8-phenyltheophylline (Sigma), L-N^6-phenylisopropyladenosine (L(−)-PIA, R-isomer), D-N^6-phenylisopropyladenosine (D(+)-PIA, S-isomer) (RBI); 5'-N-ethylcarboxamidoadenosine (NECA) was a gift from Byk Gulden, Konstanz, F.R.G.

Results

Data were obtained from experiments on eight cats.

Adenosine and adenosine analogues

NECA (4.2×10^{-12}–4.2×10^{-8} mol; 0.001–10 μg) increased carotid chemosensory discharge when injected i.c. This stable adenosine analogue caused a dose-related chemoexcitation (e.g. Figure 1) which was long-lasting, with discharge remaining elevated for several minutes after the injection and gradually returning to control levels 5–10 min after the high doses of NECA. In contrast, the dose-related chemoexcitation evoked by adenosine (3.8×10^{-11}–1.9×10^{-7} mol; 0.01–50 μg i.c.) only lasted for about 20 s, as we found in our previous experiments (McQueen & Ribeiro, 1981; 1983).

ED_{50} values were determined for NECA in three experiments by measuring the discharge during the 15 s post-injection period and expressing it as a percentage change from the pre-injection (control) frequency for each dose. The mean ED_{50} (± s.e.mean) was 2.4 ± 0.7 nmol NECA injected i.c. For comparison, the mean ED_{50} value for adenosine was 3.0 nmol (calculated from data obtained during the 15 s period following i.c. injection, shown in Figure 2 of McQueen & Ribeiro, 1981; $n = 8$).

Although less potent than adenosine as chemoreceptor stimulants, L-PIA and D-PIA (2.7×10^{-11}–2.7×10^{-7} mol; 0.01–100 μg i.c.) were both able to increase chemosensory discharge. The mean ED_{50} for L-PIA during the 15 s post-injection was 135 ± 12 nmol ($n = 2$) and a comparison between the L and D isomers in the same experiment showed L-PIA to be slightly more potent, by a factor of two, in stimulating the carotid chemoreceptors.

Injection of the drug vehicle used for the highest dose of PIA (4% DMSO in saline) had no effect on

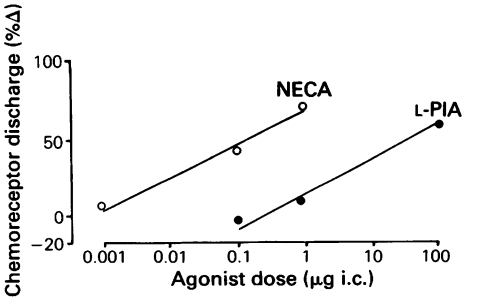

Figure 1 Dose-response data for 5'-N-ethylcarboxamidoadenosine (NECA) (○) and L-N^6-phenylisopropyladenosine (L-PIA) (●) obtained in the same experiment. Doses are plotted on a $\log_{10}$ scale against chemoreceptor response, expressed as the percentage change from pre-injection frequency of chemoreceptor discharge in the 15 s post-injection period (% Δ). Lines were fitted to the data by the least squares method and used for calculating the ED_{50} values.

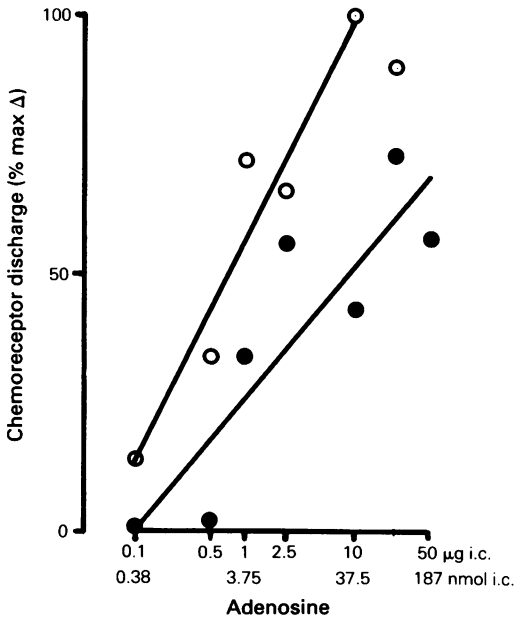

Figure 2 Log_{10} dose-response lines for adenosine obtained before (○) and during (●) infusion of 8-phenyltheophylline (8-PT, 10 μg min^{-1} i.c.) in a single experiment. The change (from pre-injection frequency) in chemosensory discharge during the 5 s post-injection period was measured for each dose, and the responses are expressed as a percentage of the maximum increment obtained before infusing 8-PT (% max Δ). Straight lines were fitted to data (except for supra-maximal value) by the method of least squares, and used for calculating ED_{50} values.

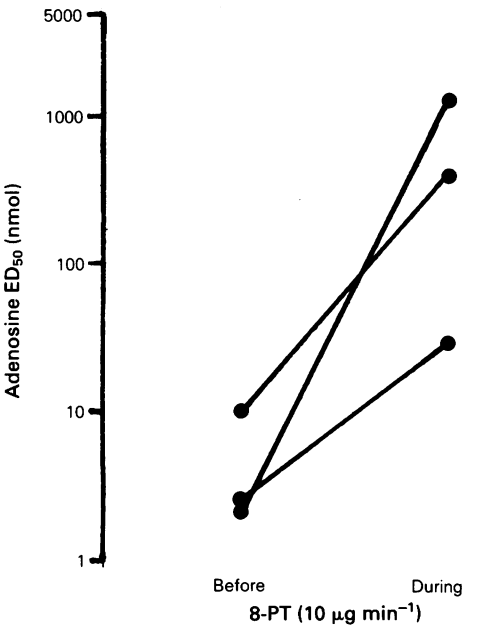

Figure 3 ED_{50} response data for adenosine, based on the 5 s post-injection period, obtained before and during infusion of 8-phenyltheophylline (8-PT, 10 μg min^{-1} i.c.). The lines join data obtained from individual experiments, and the ED_{50} values are plotted on the ordinate scale using a logarithmic scale.

chemosensory discharge or on the responsiveness of the chemoreceptors to the stimulant action of sodium cyanide (51 nmol, injected i.c.). A comparison of NECA with L-PIA in the same experiments confirmed that NECA is a much more potent chemoreceptor stimulant than L-PIA (e.g. Figure 1).

Effects of alkylxanthines

In two experiments we infused theophylline (10 μg min^{-1} i.c.) and obtained a reduction of the chemoexcitation caused by NECA. The dose-ratio for NECA, measured at the ED_{50}, averaged 14 during theophylline infusion in the two experiments.

Subsequently we decided to use the more potent adenosine receptor antagonist, 8-phenyltheophylline (8-PT; 10 μg min^{-1} i.c.) which has even less activity than theophylline as a phosphodiesterase inhibitor. For these experiments, the change in chemosensory discharge occurring during the 5 s post-injection period following adenosine injection was measured in order to minimize the possibility of changes in chemosensory discharge, secondary to actions outside the carotid body, influencing the response. The ED_{50} was expressed in terms of the half-maximal response to adenosine (see Figure 2) and values from three experiments are shown in Figure 3. It was found that infusion of 8-PT antagonized the increase in chemoreceptor discharge evoked by adenosine in each experiment, the mean dose-ratio calculated for the ED_{50} being 155.

Background (spontaneous) chemosensory discharge during air-breathing was averaged over six tests (approx 30 min) before infusion of 8-PT, and for six tests during infusion of 8-PT. Values obtained in the three experiments (one multiple-chemoreceptor, two singe unit recordings) were 12.8 ± 2.0, 2.3 ± 0.2 and 2.6 ± 0.2 ct s^{-1} before 8-PT, and the corresponding values during 8-PT were 9.8 ± 1.3, 1.7 ± 0.2 and 2.3 ± 0.3 ct s^{-1}. There was thus a tendency for discharge to be slightly reduced during infusion of 8-PT, but the differences between the means were not statistically significant in any of the three experiments ($P > 0.05$; Wilcoxon).

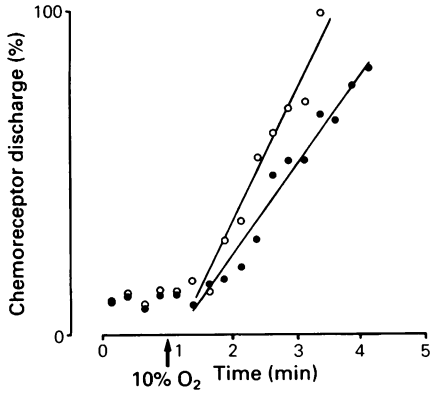

Figure 4 Responsiveness of carotid body chemoreceptors to a hypoxic stimulus (switching from ventilating with air to 10% O_2: 90% N_2 at the arrow) before (○) and during (●) an infusion of 8-phenyltheophylline (8-PT, 10 $\mu g\,min^{-1}$ i.c.). Chemosensory discharge was averaged over consecutive 15 s periods and expressed as a percentage of the steady-state discharge frequency (= 100%) measured during the pre- 8-PT state. Straight lines were fitted to the data by the method of least squares.

Influence of 8-PT on responsiveness of chemoreceptors to hypoxia

Ventilating the animal with a hypoxic gas mixture (10% O_2: 90% N_2) instead of room air increased chemosensory discharge, as illustrated in Figure 4. Experiments in two animals showed that responsiveness to hypoxia, in terms of both the slope of the line relating discharge to duration of hypoxia and the steady state discharge (= 100%) on ventilating with 10% O_2, was reduced during infusion of 8-PT 10 $\mu g\,min^{-1}$. Chemoexcitation evoked by adenosine was substantially reduced during the 8-PT infusions (see above).

Discussion

The results obtained with adenosine analogues show that the rank order of potency as chemoreceptor stimulants was NECA > adenosine ➤ L-PIA. Calculations based on data from a previous study in which we used 2-chloroadenosine (McQueen & Ribeiro, 1983) gave an ED_{50} of 33 nmol for this analogue, which ranks it between adenosine and L-PIA as a chemoreceptor stimulant. The finding that NECA was much more potent that L-PIA and that there was only a slight difference in potency between the stereoisomers of PIA, is compatible with the involvement of an A_2 type of receptor (see Introduction) in the chemoreceptor-stimulant action of these analogues. There are problems associated with using agonists to characterize pharmacological receptors, particularly when working on a preparation *in vivo*, so we can only suggest, tentatively, that these adenosine receptors in the cat carotid body are of the A_2 subtype.

Our results do not provide direct evidence concerning the location of the adenosine receptors within the carotid body. The possibilities are: cells (glomus, sustentacular), nerve terminals (sensory, motor), blood vessels, or a combination of these elements of the receptor complex. We have previously cited indirect evidence which makes it unlikely that the effects of adenosine arise from direct activation of sensory axons (McQueen & Ribeiro, 1983). The rapid response to adenosine and adenosine analogues makes it unlikely that actions on blood vessels are involved. This is because it takes a finite time for changes in carotid body blood vessels to be reflected in flow changes, and for such changes in blood flow to influence chemosensory discharge. In fact vasoactive drugs often have surprisingly little effect on chemosensory discharge in this preparation (see McQueen, 1983). By a process of elimination it would appear that cells are a likely location for the adenosine receptors, but it will be necessary to establish the site(s) of these receptors by using techniques such as radioligand binding/autoradiography and iontophoresis in slice preparations/cultured cells. One could speculate that, by analogy with the results obtained by Henon & McAfee (1983) on the rat ganglia, adenosine may facilitate transmission during repetitive firing, but biophysical experiments will be needed to investigate whether adenosine is acting directly or indirectly (affecting the release of other substances) on the chemoreceptors.

The A_2- or R_a-receptor is situated externally on cell membranes and, according to the classification of Londos & Wolff (1977), would be expected to increase adenosine 3′:5′-cyclic monophosphate (cyclic AMP) formation when activated. Indeed, in the cat, dibutyrylcyclic AMP mimics the excitatory action of adenosine on chemosensory discharge (Ribeiro & McQueen, 1983). There have not been any detailed studies relating to the effects of adenosine on cyclic AMP levels in the carotid body, but Mir *et al.* (1983) did not find any significant increase in carotid body cyclic AMP levels following intraperitoneal administration of the stable adenosine analogue 2-chloroadenosine to rats. However, these authors admit that maximal responses may not have occurred at the time the rats were killed (1.5 min post-injection). Although it might be anticipated that adenosine via A_2-receptors, would increase cyclic AMP levels in the cat carotid body, it should be borne in mind that A_2-adenosine receptors need not necessarily be involved in activating adenylate cyclase (see Stone, 1984). Whether or not cyclic AMP levels are in fact affected by adenosine in the cat carotid body must await

biochemical investigation.

It is of interest that in anaesthetized rats adenosine and its analogues increase respiration (Monteiro & Ribeiro, 1985), and this action appears to be mediated via carotid body chemoreceptors. Adenosine also stimulates respiration in man, and this effect has been attributed to carotid body chemoreceptor stimulation (Watt & Routledge, 1985). In rats the rank order of potency as respiratory stimulants (NECA > 2-chloroadenosine > L-PIA > D-PIA) was also characteristic of that required for the low affinity or A_2-adenosine receptor (Monteiro & Ribeiro, 1985).

Antagonism by alkylxanthines is one of the features which distinguishes P_1-purinoceptors (includes A_1/R_i and A_2/R_a) from P_2-purinoceptors (Burnstock, 1978), since the latter are unaffected by xanthines. We were confident from differences between the effects of adenosine and those of ATP on the chemoreceptors (McQueen & Ribeiro, 1983) that adenosine was acting at a P_1- rather than a P_2-receptor, and advanced some arguments to explain away the lack of antagonism by a single injection (1 mg i.c.) of theophylline (see McQueen & Ribeiro, 1981). The present study has clarified the situation by showing that, when infused i.c., theophylline, acts as an A_2-receptor antagonist, and the more selective antagonist, 8-PT was a potent inhibitor of adenosine-induced chemoexcitation. It is probable that in our earlier studies the dose of theophylline and the method of administration were inappropriate for demonstrating antagonism at the A_2-adenosine receptors.

It has been shown in a number of tissues (e.g. heart, brain) that hypoxia can release considerable amounts of adenosine (e.g. see Berne, 1980). In the present study we found that the adenosine receptor antagonist 8-PT reduced the responsiveness of carotid chemoreceptors to hypoxia. This could be taken as indirect evidence for the involvement of adenosine, released in the carotid body during hypoxia, in chemosensory excitation, acting directly, or as a modulator. The fact that discharge during air-breathing was not significantly affected by 8-PT may mean that adenosine does not contribute to the excitation process under normoxic/normocapnic conditions. Dipyridamole, a compound that prevents adenosine uptake by the cells, increases carotid body chemoreceptor discharge in the cat under normoxic conditions (McQueen & Ribeiro, 1983), which suggests that increases in endogenous levels of adenosine cause chemoexcitation.

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