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On the specificity and type of receptor involved in carotid body chemoreceptor activation by adenosine in the cat

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

2 Intracarotid injections of adenosine 5′-triphosphate (ATP) (1–100 μg i.c.) caused a dose-related increase in chemosensory discharge which was delayed in onset.

3 The adenosine uptake inhibitor dipyridamole potentiated the chemoexcitatory effects of injected adenosine and ATP.

4 The stable ATP analogue α-β-methylene ATP (10–100 μg i.c.) depressed chemoreceptor discharge, which suggests the presence of a P_2-purinoceptor in the carotid body, and provides evidence that the chemoexcitatory effect of ATP results from its hydrolysis to adenosine 5′-phosphate (AMP)/adenosine.

5 Adenine, inosine, guanosine, cytidine and uridine had no appreciable effect on chemoreceptor discharge.

6 The adenosine R-site agonists 2′-chloroadenosine and N^6-methyladenosine had chemoexcitatory effects which were similar to those of adenosine, whereas the P-site agonist 2′-deoxyadenosine had no appreciable effect on discharge.

7 We conclude that the adenosine receptor in the cat carotid body has some of the characteristics of an R-site receptor according to the classification of Londos & Wolff (1977).

Introduction

Adenosine excites the carotid body chemoreceptors (McQueen & Ribeiro, 1981) and so too does adenosine triphosphate (ATP) (Jarisch, Landgren, Neil & Zotterman, 1952; Dontas, 1955; Anichkov & Belen'kii, 1963; Ribeiro & McQueen, 1983) being approximately equipotent in its chemoexcitatory actions with adenosine, the parent nucleoside (Ribeiro & McQueen, 1983). This raises the question of whether it is ATP itself, or the metabolite adenosine, which excites the carotid body chemoreceptors and we decided to investigate the matter by studying the effects on the chemoreceptors of stable analogues of ATP, such as α-β-methylene ATP, which are not metabolized to adenosine.

We also undertook the characterization of the adenosine receptors which are responsible for the chemoexcitation. Two types of receptive site for adenosine have been postulated by Londos & Wolff (1977); one at which agonist activity is favoured by an unsubstituted ribose moiety (R-type), the other at which selective activation is associated with an unsubstituted purine ring (P-type). The effects of various adenosine analogues which are regarded as being selective agonists at either the P- or R-sites were studied on the chemoreceptors, as were substances such as adenine, purine nucleosides and pyrimidine nucleosides in order to test the specificity of the receptors for adenosine.

Methods

Experiments were performed on 23 cats weighing between 2.8 and 4.4 kg, median weight 3.2 kg. They

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were 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 for most of the experimental procedures have been given previously (McQueen, 1977) and only a brief description follows.

The lingual and superior thyroid arteries ipsilateral to the sinus 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 femoral artery. Electrical activity of chemoreceptors (1–6 units) was recorded from filaments of the peripheral end of a sectioned sinus nerve, stored on FM tape, passed through a pulse height (window) discriminator and quantified with the aid of a microcomputer (Commodore 3032). In the majority of experiments the ganglioglomerular (sympathetic) nerves were cut.

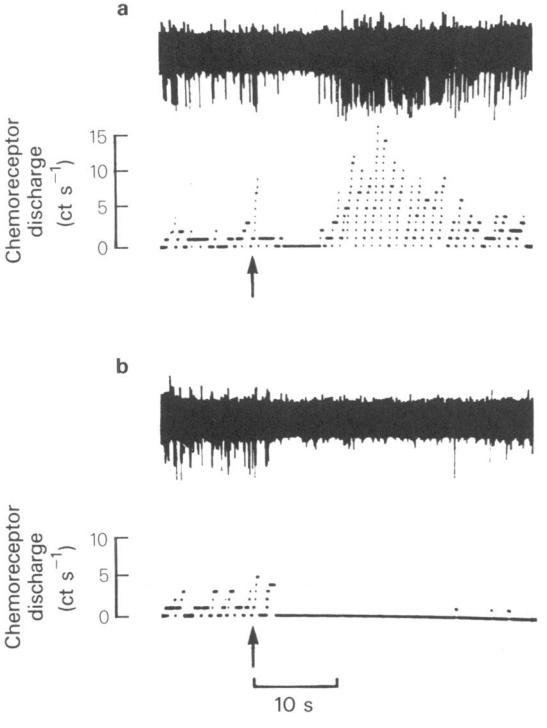

Figure 1 Recording of chemoreceptor discharge illustrating (a) the delayed excitation evoked by an injection (arrow) of ATP (100 μg i.c.) which contrasts with (b), the depression of discharge caused by injecting the same dose of α-β-methylene ATP. The lower part of each panel is the output from a pulse-height discriminator set at a fixed level to count the action potentials occurring during 1 s intervals, each step representing a single spike. The injections lasted 2 s and caused an immediate transient increase in discharge, which was also obtained when the drug vehicle was injected.

Drugs were dissolved in either modified Locke solution (McQueen, 1977) or 0.9% w/v aqueous sodium chloride solution, except for dipyridamole (500 μg ml^{-1}) which was initially dissolved in 25% alcohol:75% polyethyleneglycol. Drug injections were made in a volume of 0.1 ml into the lingual catheter (except for adenosine (100 μg and above) and adenosine analogues (100 μg) which were in 0.2 ml) 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) and lasted for 5–10 min; the catheter dead space was 0.2 ml.

Statistical analysis

The significance of differences between means was calculated using Student's paired or unpaired *t* test; *P* values of 0.05 or less were taken as being statistically significant.

Drugs

The drugs used were: sodium pentobarbitone, gallamine triethiodide (May & Baker), adenosine, adenosine triphosphate (ATP), α-β-methylene ATP, β-γ-methylene ATP, 2′-deoxyadenosine, 3′-deoxyadenosine, N^6-methyladenosine, 2′-chloroadenosine, adenine, inosine, guanosine, cytidine, uridine (Sigma), dipyridamole (Boheringer Ingelheim).

Results

Effects of ATP and ATP analogues

Injections of ATP (1–100 μg intracarotid (i.c.)) caused a dose-related increase in chemosensory discharge (see Figures 1, 2 and 3) after a delay of 2–8 s. In view of the delay to onset of the effect, we performed experiments to investigate the possibility that chemoexcitation resulted from actions of a metabolite of ATP rather than the parent compound.

Accordingly, experiments were performed in which the effects of ATP analogues were studied. The actions of α-β-methylene ATP, a compound which cannot be metabolized to adenosine, were compared with ATP. The results obtained are illustrated in Figures 1, 2 and 3 which show that, in contrast to the excitatory effect of ATP, injection of α-β-methylene ATP caused a dose-related decrease in chemosensory discharge that was associated with an increase in arterial blood pressure; ATP had little or no effect on blood pressure. Another ATP

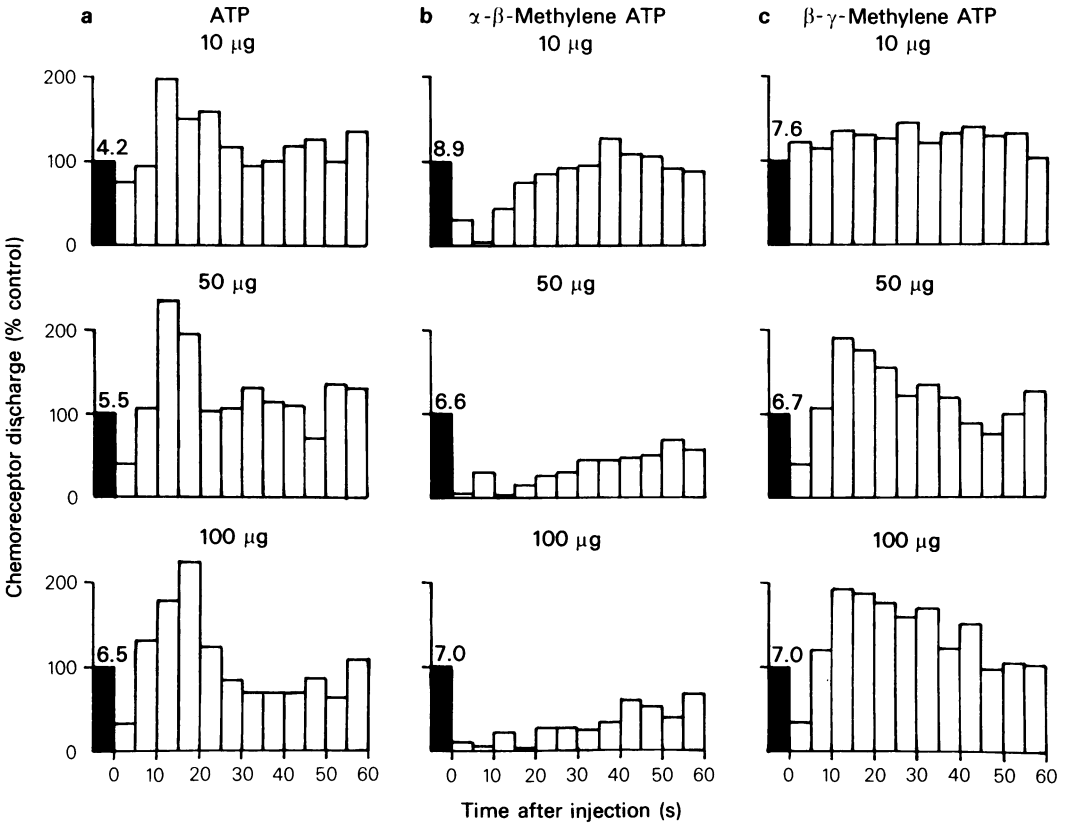

Figure 2 Quantitative comparison of the effects on spontaneous chemoreceptor discharge after injecting 10, 50 and 100 μg of (a) ATP, (b) α-β-methylene ATP and (c) β-γ-methylene ATP into the carotid artery in the same experiment. Discharge was averaged over 5 s intervals and expressed as a percentage of the pre-injection frequency (frequency shown in ct s^{-1} above solid columns which represent 100%).

analogue, β-γ-methylene ATP, which can undergo metabolism to adenosine 5'-phosphate (AMP) and/or adenosine, caused chemoexcitation which was qualitatively similar to the effect of ATP (Figures 2 and 3) and had no appreciable action on blood pressure.

Dipyridamole

The effects of dipyridamole, an adenosine uptake blocker, were studied and it was found that i.v. infusion (50 μg min^{-1}) caused a slight increase in spontaneous chemoreceptor discharge during the period of infusion. The log dose-response curve to adenosine was shifted upwards to the left during the dipyridamole infusion (Figure 4).

Intracarotid infusion of dipyridamole, also at 50 μg min^{-1}, caused a more marked increase in spontaneous discharge. During the i.c. infusion of dipyridamole the increases in discharge evoked by injections of adenosine and of ATP (1 μg) were greatly potentiated (Figure 5).

Specificity for adenosine

A number of nucleosides have actions on neurotransmission (e.g. Phillis, Edstrom, Kostopoulos & Kirkpatrick, 1979), so it was considered of interest to evaluate the effect of some of these substances on the carotid body chemoreceptor activity in order to explore the specificity of the adenosine receptor. Figure 6 summarizes results from 2 experiments in which a metabolite of adenosine, adenine, and the purine nucleosides inosine and guanosine, as well as the pyrimidine nucleosides cytidine and uridine were tested. The effects were compared with those of adenosine itself. As can be seen neither adenine (10–100 μg) nor the nucleosides inosine, guanosine, cytidine or uridine caused any substantial increases in the spontaneous chemoreceptor discharge, whereas adenosine increased discharge.

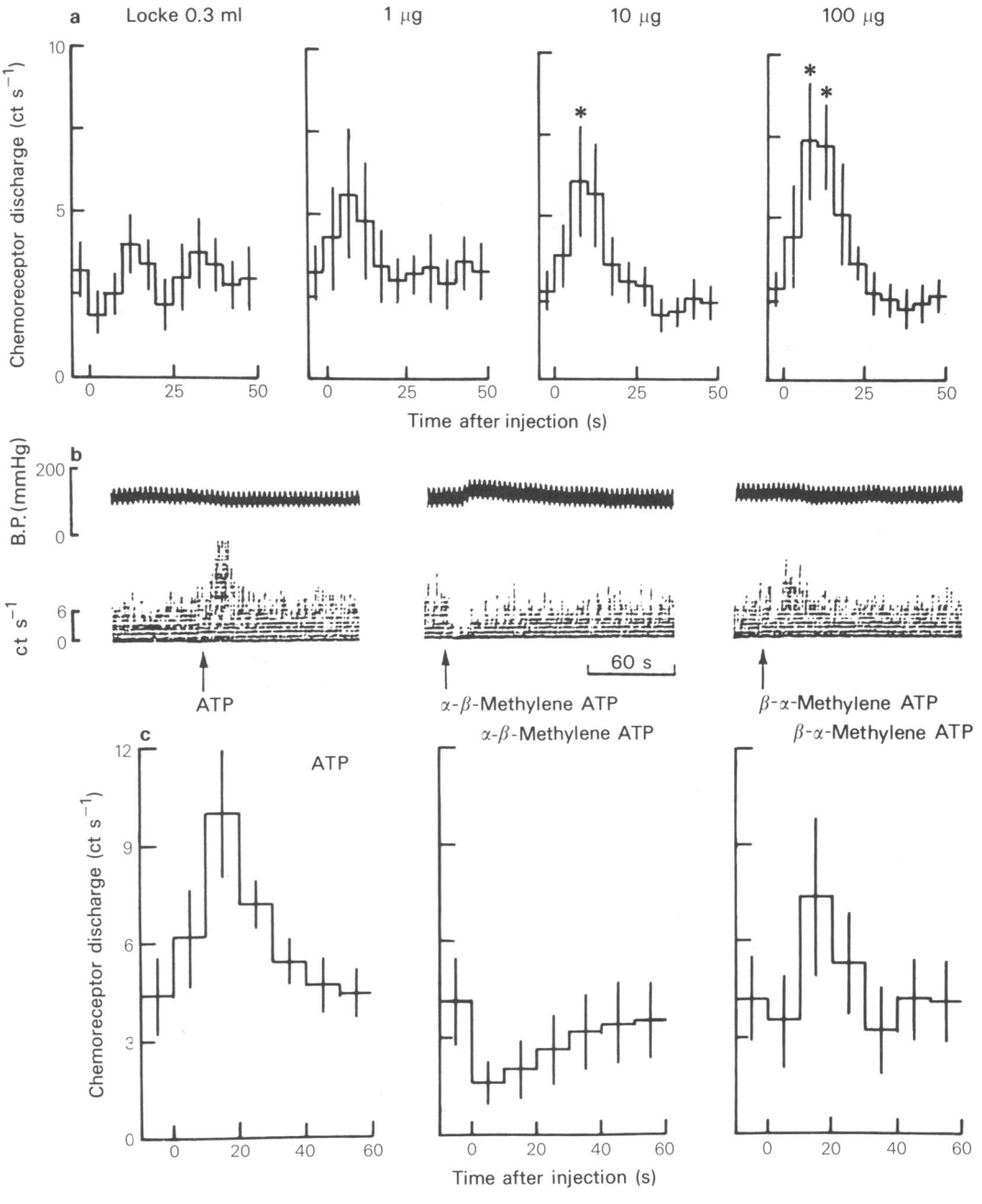

Figure 3 (a) Effects on spontaneous chemoreceptor discharge of injecting Locke solution (0.1 ml + 0.2 ml wash) and ATP 1, 10 and 100 μg i.c. Discharge (ct s^{-1}) was averaged over 5 s periods following the injection and is shown as the mean from 6 experiments ±s.e.mean. * $P<0.05$ (paired $t$test – comparison with Locke solution). (b) Comparison of the effects of injecting (arrow) 100 μg of non-hydrolysable ATP analogues α-β-methylene ATP and β-γ-methylene ATP with ATP on chemoreceptor discharge and on blood pressure showing the marked difference between α-β-and β-γ-methylene ATP on both parameters. (c) Pooled data from 4 experiments showing quantitatively the effects of these ATP analogues and ATP on chemoreceptor discharge which was averaged over 10 s periods following the injection of 1n00 μg i.c.

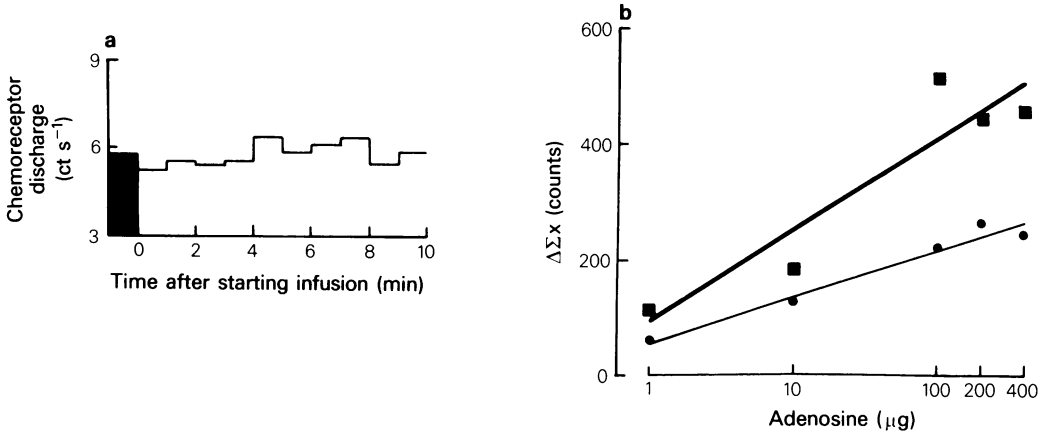

Figure 4 The effect on spontaneous chemoreceptor discharge k(ct/s^{-1}) of infusing dipyridamole (50 μg min^{-1} i.v.) (a). The solid column represents the pre-infusion control discharge frequency. (b) shows log dose-response lines for adenosine injected before (●) and during (■) infusion of dipyridamole (50 μg min^{-1} i.v.). Chemoreceptor responses are expressed as the mean $\Delta\Sigma x$ calculated as the response during the 30 s following injections $\Delta\Sigma x = \Sigma x$(response) $- \Sigma x$(control). Σx(control) = x, (control) × t (response duration in s). x = average discharge in ct s^{-1}. Pre-injection control discharge alues (ct s^{-1}) were: adenosine 1 μg 2.7 before; 2.6 during infusion; 10 μg – 2.0, 2.5; 100 μg – 3.8, 3.3; 200 μg – 3.4, 4.5; 400 μg – 5.8, 4.8. Lines were fitted to the data by the least squares method.

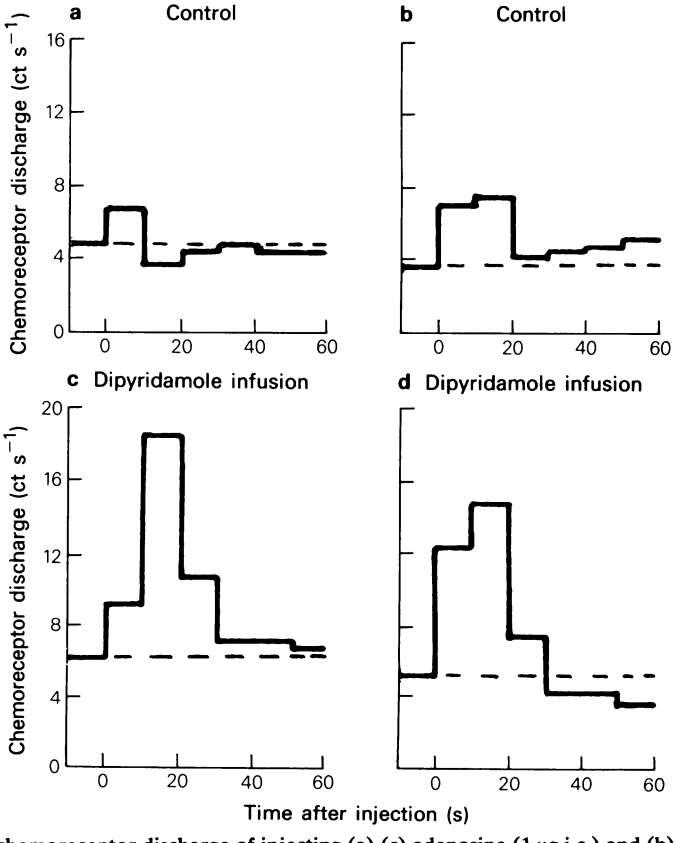

Figure 5 Effects on chemoreceptor discharge of injecting (a),(c) adenosine (1 μg i.c.) and (b),(d) ATP (1 μg i.c.) before and during an infusion of dipyridamole (50 μg min^{-1} i.c.). Discharge (ct s^{-1}) was averaged over 10 s periods. The dashed lines represent the pre-injection averaged discharge.

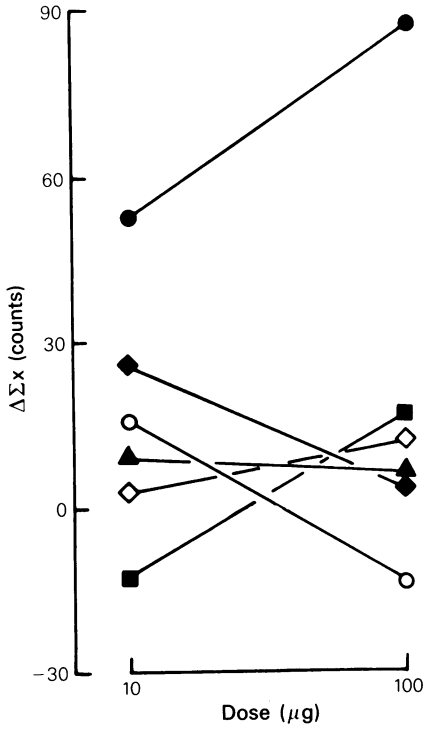

Figure 6 Comparison between the responses of chemoreceptors during the 10–30 s post-injection period to adenosine (●), adenine (○), inosine (▲), guanosine (◆), cytidine (◇) and uridine (■) in 2 cats. $\Delta\Sigma x$ has the same meaning as in Figure 4. Pre-injection control discharge values (ct s^{-1}) for 10 and 100 μg respectively were: adenosine 3.4, 3.0; adenine 2.3, 2.1; inosine, 3.5, 3.2; guanosine, 6.3, 7.8; cytidine 3.1, 2.7; uridine 1.5, 1.2.

Adenosine analogues

The responses of chemoreceptors to adenosine analogues were investigated in 5 experiments. Results obtained from one experiment, in which the effects of the analogues were studied at 2 different doses (10 and 100 μg i.c.), are shown in Figure 7, as are results from 4 experiments in which only the higher dose was used. These demonstrate that N^6-methyladenosine and 2′-chloroadenosine, regarded as R-site agonists, increased spontaneous discharge in a manner similar to that of adenosine. The compound 3′-deoxyadenosine, which can affect both R- and P-sites, but has higher affinity for the former, also increased chemoreceptor discharge. In contrast, 2′-deoxyadenosine, which is an adenosine agonist selective for the P-site had little or no effect on spontaneous discharge.

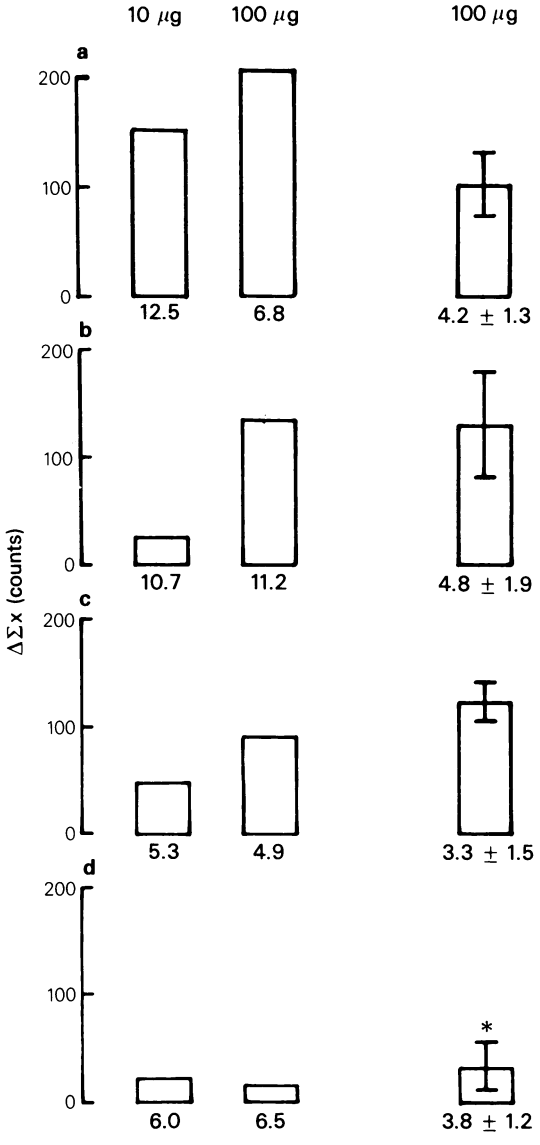

Figure 7 Response of carotid chemoreceptors to injecting 10 μg (first column) and 100 μg (second column) of the adenosine analogues (a) 2′-chloroadenosine, (b) N^6-methyladenosine, (c) 3′-deoxyadenosine and (d) 2′-deoxyadenosine illustrating the dose-dependent nature of the response to the first 3 of the 4 compounds. The third column represents pooled data from 4 further experiments in which the effects of injecting 100 μg of each adenosine analogue were compared with the response to 100 μg of adenosine. Results are expressed as the mean increase in discharge above background activity (i.e. $\Delta\Sigma x \pm$ s.e.mean (see Figure 4) during the 10–40 s period following the injection. Background or spontaneous period discharge frequency is shown below each column. Adenosine caused an increase of 170 ± 40 counts, average background discharge 3.0 ± 1.1 ct s^{-1} ($n = 4$). *$P < 0.05$ (comparison with any of the other responses by paired t test).

Discussion

The present results confirm the presence of an adenosine receptor in the cat carotid body (McQueen & Ribeiro, 1981); activation of this receptor causes an increase in chemosensory discharge. Londos & Wolff (1977) postulated the existence of membrane bound adenosine receptors with an internally located P-site and an externally located R-site. The P-site is activated by 2'-deoxyadenosine and the R-site selectively affected by agonists such as N^6-methyladenosine and 2'-chloroadenosine. The present results are compatible with an R-site adenosine receptor being present in the carotid body since the R-site agonists (N^6-methyladenosine, 2'-chloroadenosine) caused effects very similar to those of adenosine, whereas the P-site agonist (2'-deoxyadenosine) had no appreciable effect on chemosensory discharge. However, in many systems the action of adenosine can be blocked effectively by theophylline, whereas in the chemoreceptors it does not seem to be antagonized (McQueen & Ribeiro, 1981). This could mean that a different type of receptor is involved in the response of the chemoreceptors to adenosine, but it should be noted that some adenosine receptors are relatively insensitive to xanthines (Daly, 1983), and complications may arise from the use of theophylline *in vivo* because of the adenosine uptake-blocking and phosphodiesterase-inhibiting properties of the drug. Neither adenine, the purine nucleosides inosine and guanosine, nor the pyrimidine nucleosides cytidine and uridine had any appreciable effect on chemosensory discharge.

The adenosine receptor appears to be externally located since the adenosine uptake antagonist, dipyridamole, potentiated the chemoexcitation evoked by injected adenosine. This further supports the presence of an R-site adenosine receptor in the carotid body, since it is a characteristic of these sites that they are externally located (Daly, Bruns & Snyder, 1981). However, our results do not allow us to establish whether the adenosine receptor is associated with sensory nerve terminals, glomus type I and/or type II cells, blood vessels, or with all these structural components of the sensory complex. It seems unlikely that the effect of adenosine results from direct activation of the sensory axons because adenosine does not change either the amplitude or duration of the compound action potential in frog-sciatic nerve (Ribeiro & Dominguez, 1978) or of action potentials of unmyelinated axons in the brain (Stone, 1981).

ATP did show effects which were similar to those of the nucleoside adenosine, but the nucleotide differed in that there was a delay to onset of the response. This raised the possibility that this effect of ATP on the chemoreceptors depends on its undergoing hydrolysis to AMP and/or adenosine. Experiments with the stable ATP analogue α-β-methylene ATP showed clearly that in this stablized form ATP does not activate chemoreceptors, and indeed it actually depressed discharge. The analogue β-γ-methylene ATP, which can be metabolized to AMP/adenosine, had effects similar to those of adenosine. Therefore, we can conclude that the chemoexcitatory effects associated with ATP are probably attributable to one of its metabolites, possibly adenosine.

The finding that a stable analogue of ATP causes chemoinhibition, whereas adenosine consistently causes chemoexcitation, could mean that in addition to P_1-purinoceptors (Burnstock, 1978), which include both the adenosine P- and R-sites of Londos & Wolff (1977), P_2-purinoceptors, which are activated by ATP, are also present in the carotid body.

According to Londos & Wolff (1977), activating the adenosine P-site would be expected to inhibit the activity of adenylate cyclase, so causing a decrease in levels of cyclic AMP, whereas activation of the R-site is usually associated with activation of adenylate cyclase and would be expected to alter cyclic AMP levels. Whether or not this applies in the cat carotid body remains to be investigated and would help in the classification of the site. The physiological significance of adenosine receptors in this structure remains to be established.

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