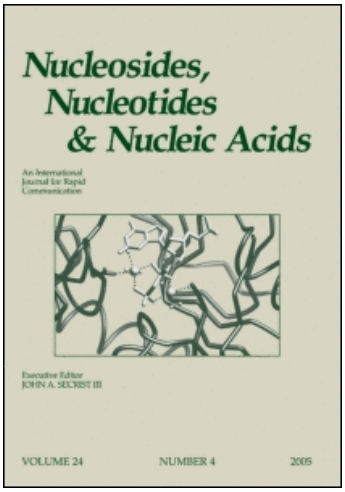

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ON THE ADENOSINE RECEPTOR INVOLVED IN THE EXCITATORY ACTION OF ADENOSINE ON RESPIRATION: ANTAGONIST PROFILE

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Abstract. The use of xanthines to characterize the adenosine receptor involved in excitation of respiration revealed that XCC, PD 115,199 and XAC have much higher affinities than DPCPX followed by PACPX, DPX and 8-PT, which is compatible with an A_2 type of adenosine receptor.

INTRODUCTION

In terms of adenosine agonists the adenosine receptor, involved in the excitation of respiration mediated through carotid body chemoreceptors in the rat, has an agonist profile with 5'-N-ethylcarboxamidoadenosine (NECA) > 2-chloroadenosine (CADO) > R-N^6-phenylisopropyladenosine (R-PIA)[1]. This profile was also observed in experiments recording chemoreceptor sensory activity from the cat carotid sinus nerve[2].

Little controversy has emerged on this agonist profile and the classification of this receptor as an A_2 subtype[3]. Nevertheless, antagonist criteria, where possible, is the first choice criteria to classify receptors. The advent of several antagonists[4] some of them [e.g. 1,3-dipropyl-8-cyclopentyl-xanthine (DPCPX)] being very selective for the A_1-adenosine receptor either in functional[5] or binding studies[6-12], prompted us to investigate the affinities of the different antagonists, (1,3-dipropyl-8-(4-(2-aminoethyl)amino)carbonylmethyloxyphenyl)xanthine (XAC), 1,3-dipropyl-8-(carboxymethyloxyphenyl)xanthine (XCC), [benzenesulfonamide, N-(2-(dimethylamino)-ethyl)-N-methyl-4-(2,3,6,7,-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl] (PD 115,199), 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine (PACPX), 1,3-diethyl-8-phenylxanthine (DPX) and 8-phenyltheophylline (8-PT) for the adenosine receptor involved in the carotid body excitation of respiration.

These antagonists have been used by our group to characterize the presynaptic inhibitory (A_1) receptors at the rat neuromuscular junction and at the hippocampus[5] as well as the presynaptic inhibitory (A_3)

receptor at the frog neuromuscular junction[13]. The K_i value for DPCPX at the A_1 receptor in the rat was 0.54 nM in the neuromuscular junction and 0.44 nM in the hippocampus. XCC has a K_i of 10 nM in the rat neuromuscular junction and 5.4 nM in the hippocampus; the K_i values of XAC were 11 nM in both preparations[5]. In the frog neuromuscular junction (A_3 receptor) the K_i of DPCPX was 35 nM, the K_i of XAC was 23 nM, and that of XCC was 1905 nM[13]. It appears, therefore, that by using antagonists it is possible to define different antagonist profiles for adenosine receptors.

In the present work we studied the affinities of these antagonists having in mind that we are using an in vivo preparation.

METHODS

Experiments were performed on Wistar rats weighing approximately 400 g, anaesthetized with sodium pentobarbitone (60 mg/Kg i.p., supplemented as required during the experiments) vagotomized and breathing room air spontaneously. The respiratory airflow ($\dot{V}$), respiratory frequency (f), tidal volume (V_T), arterial blood pressure (BP) and heart rate (HR) were recorded continuously during the experiments. The respiratory minute volume ($\dot{V}_E$) was calculated as the product of V_T and f. Intracarotid (i.c.) injections of adenosine in a volume of 0.1 ml, and infusions of xanthines at a rate of 0.5 ml/min, during 1 min were made through a catheter introduced via the external carotid artery, with its tip positioned into the common carotid artery just below the bifurcation. The intervals between adenosine injections were at least 5 min. Two cumulative dose-response curves for adenosine (i.c.) were performed in each rat: one before and the other starting 10 min after the end of i.c. infusions of the xanthines. Only one xanthine was tested per animal. As a control for the experiments with xanthines, dose-response curves for adenosine were performed in one animal before and after an i.c. infusion of DMSO in a concentration (10% v/v) used to obtain the solution of PACPX. This solution contained the highest concentration of DMSO used to solve xanthines, but did not markedly change the dose-response curves obtained for adenosine. Control values for V_T, f, BP and HR correspond to the mean values measured in a period of 24 s immediately before drug administration. After drug i.c. administration the values of V_T, f, BP and HR, were taken as the maximal effects measured during the period of 24 s that followed the injections or during drug infusions, and were compared with those measured during the control. The maximal effects induced by i.c. injections of adenosine always occurred in the first 24 s that followed the end of the injections.

RESULTS

Ventilation. As a consequence of administration of adenosine (100 nmol) into the common carotid artery (i.c.) in the presence of the xanthine amine congener (XAC, 1nmol) the excitatory effect of the nucleoside on respiration was decreased (FIG. 1). This reduction was observed in both tidal volume (V_T) and in the frequency of respiration (f). Expressing the product of V_T x f as $\dot{V}_E$, as can be seen in FIG.1 the percentage increase caused by the different doses of adenosine was antagonized by XAC. The effect of XAC was dose-dependent, and the dose of 1 nmol caused a marked shift (by a factor of 17 calculated for the dose that produced 25% increase in $\dot{V}_E$) to the right in the dose-response curve obtained for adenosine. This marked shift influenced the Schild treatment of the results obtained with the different doses of XAC (see TABLE 1). The slope obtained for this xanthine was 3.63, rather high and different from the unity. During the administration of cumulative doses of adenosine in the presence of the highest dose of XAC the basal values for V_T and f, as well as their product $\dot{V}_E$, were slightly above the basal values recorded for V_T or f before adenosine administration in absence of the antagonist . No marked changes in these basal values (V_T and f) were detected with administration of XAC in low doses: 0.5 and 0.7 nmol (see legend to FIG.1).

Dose-response curves for adenosine were performed in the absence and presence of other xanthines, DPCPX, xanthine carboxylic congener (XCC) and PD115,199. The antagonistic action of both DPCPX, XCC and PD 115,199 were dose-dependent. In the case of DPCPX its affinity (K_i=120 nM) was about 5 times less than the affinity determined for XAC (K_i=23 nM). Affinities not different by a factor of two from that obtained for XAC were also observed for XCC (K_i=14 nM) and PD115, 199 (K_i=15 nM) (see TABLE 1).

Other xanthines have been used, PACPX, DPX and 8-PT, and their affinities evaluated from single experiments. These xanthines were used in single doses PACPX (50 nmol), DPX (50 nmol) and 8-PT (100 nmol), and the determination of DR_2 values showed higher values than those obtained for the other xanthines (XCC, PD 115,199, XAC or XCC) (see TABLE 1). Therefore, we did not investigate in detail the characteristics of these adenosine antagonists.

Arterial Blood Pressure and Heart Rate. The cardiovascular changes induced by intracarotid administration of adenosine and their modifications by the adenosine receptor antagonists, PD 115,199, XAC, XCC, DPCPX were studied by examining arterial blood pressure (BP) and heart rate (HR). In FIG. 2 are shown the effects of adenosine on BP before and after infusing XAC or DPCPX into the common carotid artery. It can be seen that XAC (1 nmol/min during 1 min) clearly reduced the

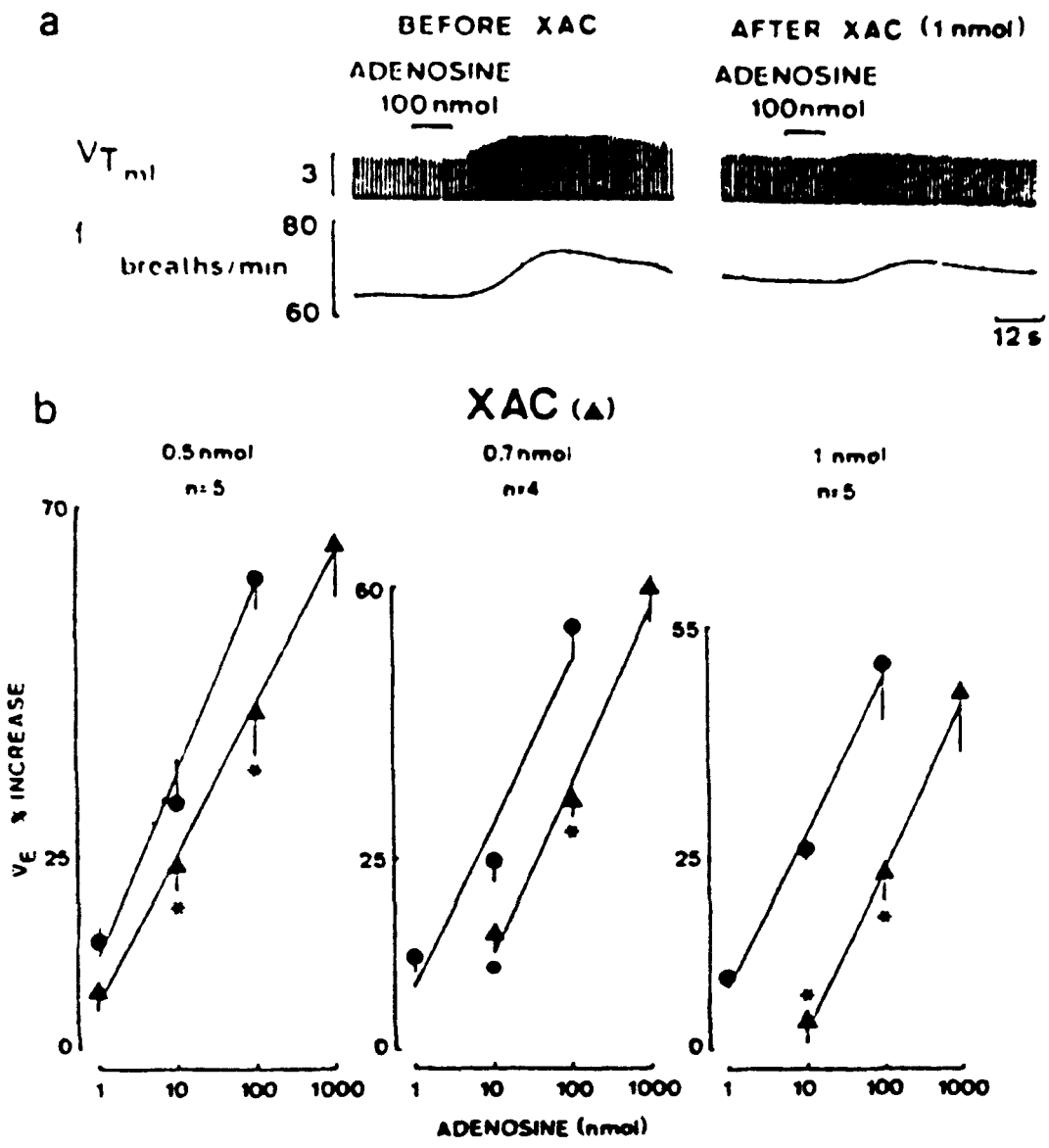

FIG. 1. Effects of the adenosine receptor antagonist (1,3-dipropyl-8-(4-(2-aminoethyl)amino)carbonylmethyloxyphenyl)xanthine (XAC) on the excitatory effect of adenosine on respiration of rats anesthetized with pentobarbitone. (a) Effect of intracarotid (i.c.) injections of adenosine (100 nmol) on tidal volume (V_T) and respiratory frequency (f) before and approximately 40 min after i.c. infusion of XAC (1 nmol/min, during 1 min). The effect of adenosine shown is the result of the fifth injection of five cumulative and consecutive injections of the nucleoside. These injections were: 0.01, 0.1, 1, 10 and 100 nmol given with intervals of at least 5 min. The excitatory effect of adenosine (100 nmol) on V_T and f disappeared within 75 s after its i.c. injection. (b) Cumulative dose-response curves for the excitatory effects of i.c. injections of adenosine on respiratory minute volume

(continued)

TABLE 1. Potency of xanthine derivatives as antagonists of the excitatory effect of adenosine on respiratory minute volume in rats.

Drug	DR_2 (nmol)	K_i (nM)[a]	slope[b]	n
XCC	0.28	14	1.53	15
PD115,199	0.30	15	1.37	12
XAC	0.5	23	3.63	13
DPCPX	2.4	120	1.3	14
PACPX[c]	6.2	310	—	1
DPX[c]	9.34	467	—	1
8-PT[c]	20.32	1016	—	1

DR_2: Dose of the xanthine derivative that produces a dose-ratio of 2 for adenosine.

[a] K_i values were estimated from DR_2 values assuming a volume of distribution of 20 ml.

[b] slope of the regression lines calculated from the Schild plot.

[c] DR_2 and K_i values were estimated using only one concentration of the xanthine in an abbreviated Schild analysis, where a slope of 1.3 was assumed for log (DR-1) on log (xanthine).

XCC: 1,3-dipropyl-8-(carboxymethyloxyphenyl)xanthine

PD115,199: benzenesulfonamide, N-(2-(dimethylamino)ethyl)-N-methyl-4-(2,3,6,7,-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)

XAC: (1,3-dipropyl-8-(4-(2-aminoethyl)amino)carbonylmethyloxyphenyl) xanthine

DPCPX: 1,3-dipropyl-8-cyclopentylxanthine

PACPX: 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine

DPX: 1,3-diethyl-8-phenylxanthine; 8-PT: 8-phenyltheophylline

FIG. 1. Continued

($\dot{V}_E$) before (●) and after (▲) i.c. infusions of XAC (0.5, 0.7 and 1 nmol). Doses of adenosine are plotted on a $\log_{10}$ scale and the effect on $\dot{V}_E$ is the maximal effect expressed as % increase in ml/min observed in the first 24 s that followed the end of the injections. 0% represents absolute values determined for the 24s that preceded the injections. Absolute values before the injections of adenosine in the absence and in the presence of XAC 0.5 nmol were respectively 158±6 ml/min and 160±3 ml/min ; in the absence and in the presence of XAC 0.7 nmol respectively 173±4 ml/min and 178±8 ml/min; and in the absence and in the presence of XAC 1 nmol respectively 163±6 ml/min and 187±6 ml/min. Each point is the average of 4 or 5 experiments and the vertical bars represent ± SEM and are shown when they exceed the symbols in size. The regression lines were calculated by the method of the least squares and the correlation coefficients ranged from 0.968 and 0.998. * $p<0.05$ (Student's paired t-test).

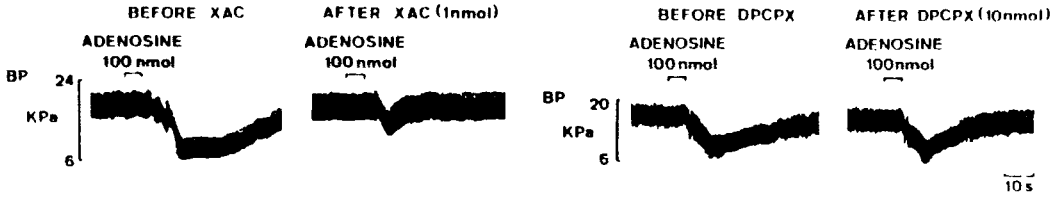

FIG. 2 Effects of intracarotid i.c. injections of adenosine (100 nmol) on arterial blood pressure (BP) before and approximately 40 min after i.c. infusion of (1,3-dipropyl-8-(4-(2-aminoethyl)amino)carbonyl-methyloxyphenyl) xanthine (XAC) (1 nmol/min, during 1 min) or 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (10 nmol/min, during 1 min) in two different rats.

amplitude of the hypotensive effect of adenosine (100 nmol) whereas DPCPX (10 nmol/min during 1 min) caused little or no effect on the amplitude of the BP responses to adenosine.

From the results of several experiments it was quantified the percentage of antagonism calculated for the adenosine antagonists: PD 115,199, XAC, XCC and DPCPX in relation to the hypotensive responses induced by adenosine (100 nmol). The most effective xanthines were PD 115,199, XAC and XCC, which in the dose of 1 nmol, all antagonized by about 25% the hypotensive effect of adenosine, whereas DPCPX has only a small antagonist action (≃15%) with the highest dose used (10 nmol). This relative position was not very different from that observed for the xanthine antagonism of the adenosine's excitatory effect on ventilation (see TABLE 1).

The antagonism by xanthines of the bradycardic effect of adenosine could not be properly investigated, since only with the highest dose of adenosine (100 nmol) some decrease in HR could be seen (11±3% decrease in HR). In the case of XAC (1 nmol) it was possible to reduce the bradycardic response to 6±0.5% decrease and in the presence of DPCPX (10 nmol) the HR decrease induced by adenosine was 7.5±1%. In contrast with almost absence of antagonism of the hypotensive effect of adenosine, DPCPX caused some antagonism in HR. This might be related to the higher affinity of this antagonist for the adenosine receptor mediating the bradycardic response (A_1) as compared with the receptor mediating adenosine's vasodilator effect (A_2).

DISCUSSION

The present results show that the affinities of the xanthines that antagonized the excitatory effect of adenosine on respiration mediated

through carotid body chemosensory activity, exhibited a relative position with XCC (K_i=14 nM), PD 115,199 (15 nM), XAC (23 nM) > DPCPX (120 nM) > PACPX (310 nM) ≧ DPX (467 nM) > 8-PT (1016 nM). This profile does not correlate with the A_1 profile [DPCPX (K_i=0.54 nM) >XCC (10 nM), XAC (11 nM), PACPX (13 nM) ≧ DPX (22 nM), 8-PT (25 nM) > PD 115,199 (57 nM)] or the A_3 profile [XAC (K_i=23 nM), DPCPX (35 nM) > 8-PT (200 nM), DPX (295 nM) > XCC (1905 nM), PACPX (2291 nM)] determined in functional studies[5 13]. The affinity profile for these xanthines, obtained in the present work, is also quite different from that of the A_1 receptor characterized as displacement of [^{3}H]-R-PIA or [^{3}H]-CHA binding to rat brain membranes. In this preparation DPCPX (K_i=0.4-0.5 nM) > XAC (1.2-3.5 nM) ≧ PACPX (2.5-6.8 nM) > PD 115,199 (8-14 nM) > XCC (50-58 nM), DPX (44-70 nM) > 8-PT (160-400 nM) [6 - 12]. The relative position of the antagonists (present work) is similar to that obtained in displacement of the binding of the relatively selective A_2-adenosine receptor agonist [^{3}H]-NECA to rat striatal brain membranes[8-10], where PD 115,199 (K_i=16 nM) ≧ XAC (24 nM) > PACPX (92 nM) > DPCPX (340 nM) > DPX (550-860 nM). These authors did not test XCC, which in present study proved to be one with high affinity for the adenosine receptor involved in excitation of respiration by the nucleoside. Comparing our results with those described[6 10 14] for the adenylate cyclase (AC) activation/cyclic AMP accumulation caused by NECA in human platelets in which the antagonist affinity profile was XAC (K_i=24-25 nM) > DPX (210-270 nM) ≧ DPCPX (330-390 nM) ≧ PACPX (440-470 nM) > 8-PT (1900-4100 nM), it was found good correlation (r=0.880, p<0.01; slope=1.038) with the profile obtained in the present investigation. The exception was XCC, which in the present study was one of the most effective xanthines, but in relation to the AC/activation, XCC is a very poor antagonist[14].

Studies <u>in vivo</u>[15] performed in rats anaesthetized with the same anaesthetic (sodium pentobarbitone) as that used in the present work, showed that in relation to the receptor, postulated as an A_2 subtype, that mediates decrease in arterial blood pressure, XCC was more potent then XAC. The DR_2 ratio for XAC/XCC obtained in the present work was 1.8 and in relation to decrease in BP[15] was 1.13, these ratios beeing not very different . A point of interest in the present results is that the percentages of antagonism observed with XAC, PD 115,199 and XCC were roughly similar in both ventilation and decrease in BP. DPCPX was much less potent than these three xanthines to antagonize adenosine excitation of ventilation. In relation to decrease in BP, DPCPX in the doses used was almost devoid of antagonism.

The estimation of the potency of the xanthine derivatives as antagonists of the excitation of respiration by adenosine was expressed as DR_2 values; the conversion of these values into molar concentrations, was made assuming that the rats used have a plasma volume of about 20 ml. The values obtained for XAC and XCC were within low nanomolar range.

This is an order of magnitude lower than the values estimated by others[15] for the antagonism of BP, and about the same order of magnitude as the K_i values calculated for XAC from in vitro studies[10 14].

The adenosine receptor operating both the excitation in respiration and decrease in BP appears to be the same A_2 type as can be inferred from their agonist profiles[1]. In the present work the antagonism of the adenosine excitatory effect on ventilation and the antagonism of the decrease caused by adenosine in BP was similar in the case of XAC, PD 115,199 and XCC, which in both situations have higher affinities than DPCPX. These findings suggest that a similar receptor, probably of the A_2 type, mediates both effects.

In the present experimental conditions the highest dose of adenosine caused only a small decrease in HR. This did not allow to perform convenient dose-response curves to know the relative affinities of the different antagonists. The highest dose of adenosine used (100 nmol) caused a decrease in HR of 11±3% and this was antagonized by both XAC and DPCPX but not by XCC or PD 155,199 , which were the most potent to antagonize the effects of adenosine on both BP and ventilation. This conforms with the idea that the decreases in BP and the decreases in HR are operated by different adenosine receptors.

Almost no data is available on the pharmacokinetics of the xanthines used in the present work. In the case of XAC it can be metabolized into XCC[16], which was the most potent compound to antagonize the excitation of adenosine on respiration. The slope of XAC (3.63) was much higher than the unity suggesting that different factors may contribute to this value. Whether its metabolism to XCC would influence the slope observed needs to be investigated. In the highest dose XAC increased ventilation, probably as a consequence of entering to the brain, where xanthines can excite respiration. Whether this is another variable that influences the slope of XAC needs to be evaluated. The antagonism by these xanthines was probably exerted at the carotid body level, since there is no evidence that XCC or XAC in low doses could easily cross the blood brain barrier[15].

DPCPX and XAC are useful tools to discriminate adenosine receptors. In the case of the A_1 DPCPX is consistently more potent than XAC either in functional or binding studies in vitro. In the case of the A_2 DPCPX is usually less potent than XAC. In the case of the A_3 DPCPX has an intermediate position and is as potent as XAC. In spite of XAC being devoid of any selectivity between the different adenosine receptors (K_i≃11-23 nM), this property might be useful when we take the affinity of XAC as a reference to compare with the affinity of DPCPX.

In relation to the other antagonists used, PD 115,199, PACPX, DPX and 8-PT, their affinities correlated with those observed for displacement of [^{3}H]-NECA in rat striatal membranes and for AC/cyclic

AMP accumulation in human platelets. In the cases of XCC it was found in the present work that its affinity was higher than that described for the A_2 receptors in human platelets.

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