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Regulation of renal adenosine A_1 receptors: effect of dietary sodium chloride

Jane A. Smith, Elaine M. Whitaker, Naciye Yaktubay, Michael J. Morton, Christopher J. Bowmer, Michael S. Yates *

School of Biomedical Sciences, Worsley Medical and Dental Building, University of Leeds, Leeds LS2 9JT, UK

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Abstract

The influence of dietary NaCl on the regulation of renal adenosine A_1 receptors was investigated in the rat. Renal membranes from rats fed on a diet low (0.04%) in NaCl showed a 46% increase in B_{max} for the binding of [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX), a selective adenosine A_1 receptor antagonist, compared to membranes from rats fed on a normal diet (0.4% NaCl). Conversely, a high NaCl diet (4.0%) resulted in a 37% decrease in B_{max}. Levels of renal adenosine A_1 receptor mRNA were 65% lower in rats on a high salt diet. Autoradiographic studies showed that, for the inner medullary collecting ducts, a low NaCl diet resulted in a 30% increase in [^{3}H]DPCPX binding with a 39% decrease noted in rats maintained on a high salt diet. The results indicate that changes in adenosine A_1 receptor density may represent a novel mechanism whereby the kidneys adapt to changes in salt load.

Keywords: Kidney; Adenosine A_1 receptor; 1,3-dipropyl-8-cyclopentylxanthine; Autoradiography; Dietary NaCl

1. Introduction

Adenosine affects various aspects of renal function; namely, renal blood flow and its distribution within the kidneys, renin secretion, glomerular filtration rate, Na^+ excretion and urine flow (Collis et al., 1994). The renal actions of adenosine are those of a paracrine agent and are mediated by different receptors, four of which have been cloned (A_1, A_{2A}, A_{2B} and A_3; Collis and Hourani, 1993). Adenosine, via stimulation of A_1 receptors, may mediate tubuloglomerular feedback, a system which regulates single nephron glomerular filtration rate by adjusting afferent arteriole tone in response to changes in luminal NaCl concentration at the macula densa (Schnermann et al., 1991). The adenosine A_1 receptor subtype also mediates the antidiuretic and antinatriuretic effects of adenosine that result from enhanced tubular Na^+ reabsorption (Balakrishnan et al., 1996; Panzacchi et al., 1997). Administration of selective adenosine A_1 receptor antagonists to healthy human subjects or to normal rats produces a diuresis and natriuresis (Balakrishnan et al., 1993; Knight et al., 1993), which suggests that adenosine, via activation of adenosine A_1 receptors, exerts a tonic influence on Na^+ excretion.

Blood volume and extracellular fluid homeostasis require regulation of Na^+ excretion in response to changes in dietary Na^+. Such regulation involves a number of control systems, for example, the renin-angiotensin system. Since adenosine, via A_1 receptors, influences Na^+ reabsorption, the renal paracrine actions of adenosine may also contribute to regulation of Na^+ excretion in response to changes in Na^+ intake. Siragy and Linden (1996), using a microdialysis technique, measured adenosine levels in renal cortical and medullary interstitial fluid of rats fed diets varying in NaCl content. Switching rats from a normal to either a low or high salt diet produced parallel changes of up to sixfold in the adenosine concentration of cortical and medullary interstitial fluids (Siragy and Linden, 1996). Whilst changes in endogenous adenosine levels per se may alter renal haemodynamics and tubular function, sustained increases or decreases in adenosine concentration might be expected to evoke changes in adenosine receptor density and distribution. Such changes in adenosine receptors could

* Corresponding author. Tel: +44-113-233-4316; fax: +44-113-233-4228.

E-mail address: m.s.yates@leeds.ac.uk (M.S. Yates)

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contribute to the adaptive changes in renal function produced by variations in salt intake. The aim of this study was to investigate the effect of dietary NaCl on renal adenosine A_1 receptors. Ligand binding, the reverse transcriptase polymerase chain reaction and autoradiography were employed to assay adenosine A_1 receptors and mRNA in kidneys of rats fed diets with varying NaCl content. A preliminary account of some of these studies has been reported (Smith et al., 1998).

2. Materials and methods

2.1. Materials

[^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX) (120 Ci mmol^{-1}) was obtained from Du Pont (Stevenage, Hertfordshire, UK). DPCPX, polyethylenimine, phenylmethylsulphonyl fluoride and adenosine deaminase (type V) were obtained from Sigma (Poole, Dorset UK) α-[^{32}P]dCTP was obtained from ICN Pharmaceuticals (Thame, Oxfordshire, UK) and oligonucleotide primers were obtained from Genosys Biotechnologies (Cambridge, UK).

2.2. Methods

2.2.1. Animal preparation

Male Wistar rats (200 to 250 g) were maintained on either a normal (0.4% w/w), low (0.04%) or high (4%) NaCl diet for 7 days. During this period, rats had free access to tap water. Body weight, and food and water consumption were monitored daily. On day 6, rats were transferred to metabolic cages and urine collected for 24 h. Following removal of kidneys on day 7 (see below), a blood sample was obtained from the inferior vena cava. Urinary and plasma Na^+ concentrations were determined by flame photometry (Corning 480). Rat diets were obtained from Special Diets Services (Wincham, Cheshire, UK). The care and use of animals was carried out according to the Code of Practice as set out by the (UK) Animals (Scientific Procedures) Act 1986.

2.2.2. Saturation isotherms and competition experiments

On day 7, rats were anaesthetised with sodium thiopentone (120 mg kg^{-1}, i.p.), both kidneys were removed from each animal and freeze clamped in liquid nitrogen. Kidneys were either processed immediately or stored at −70°C for no longer than 2 days. Renal cell membranes were prepared as described previously (Gould et al., 1997). Protein concentration in renal cell membranes and tissue sections was determined by the method of Lowry et al. (1951) with bovine albumin as the standard.

All ligand binding experiments were carried out as described by Gould et al. (1997). Briefly, renal cell membranes were preincubated at 37°C in 50 mM Tris buffer, pH 7.4, containing 0.25 mM sucrose, 0.1 mM of the protease inhibitor phenylmethylsulphonyl fluoride and 5 u ml^{-1} adenosine deaminase for 30 min. All binding experiments were performed at 4°C with 500 μg of protein suspended in 500 μl of 50 mM Tris buffer, pH 7.4.

To study the dependence of binding on ligand concentration, incubations were carried out for 5 h in the presence of 0.08 to 12 nM [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX). Nonspecific binding was determined in parallel in the presence of 1 mM theophylline. Binding was terminated by rapid filtration through Whatman GF/C glass fibre filters housed in an Ilacon Cell Harvester. Glass fibre filters were pretreated by overnight soaking in 1% v/v polyethylenimine (Bruns et al., 1983). Used filters were washed with 4×2.5 ml of ice-cold buffer, dried, transferred to scintillation vials and counted for radioactivity.

2.2.3. Reverse transcriptase polymerase chain reaction (RT-PCR)

Rats were anaesthetised with sodium thiobutabarbitone (180 mg kg^{-1}, i.p.) and both kidneys removed. Kidneys were immediately freeze clamped in liquid nitrogen and stored at −70°C until use. Total cellular RNA was isolated by the acid–guanidium thiocyanate–phenol–chloroform extraction method of Chromoczynski and Sacchi (1987) and adjusted to approximately 1 μg μl^{-1} in diethylpyrocarbonate-treated water.

Two micrograms of total RNA were reverse transcribed in a total volume of 20 μl which included: 50 mM Tris buffer (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 μM dithiothreitol, 200 u of M-MLV reverse transcriptase and 0.2 μg of oligo $(dT)_{12-18}$ primer. The reaction was carried out at 37°C for 10 min. Five microlitres of the reverse transcribed material were then subjected to PCR in a total volume of 50 μl containing 10 pmol of each primer, 50 μM dNTPs, 10 mM Tris buffer (pH 8.4), 50 mM KCl, 2 mM $MgCl_2$, 0.1% Triton X-100, 1 μCi α-[^{32}P]dCTP (specific activity; 3000 Ci mmol^{-1}) and 2.5 u of *Taq* DNA polymerase prepared according to the method of Pluthers (1993). The adenosine A_1 receptor-specific primers were 5′-CTCGCCATTGCTGTGGATCGA-3′ and 5′-GTGTGTGAGGAAGATGGCGAT-3′, designed to generate a 540 bp product and β-actin-specific primers were 5′-TTGTAACCAACTGGGACGATATGG-3′ and 5′-GATCTTGATCTTCATGGTGCTTAGG-3′, designed to generate a 740 bp product. Tubes were subjected to 30 cycles: 94°C for 40 s; 64°C for 1 min, and 72°C for 1 min. PCR for β-actin employed a 'touchdown' PCR protocol (Don et al., 1991) with the primer annealing temperature ranging from 69°C to 61°C. After PCR, a 10 μl aliquot of each sample was electrophoresed on a 1% agarose gel using Tris–acetate–EDTA buffer (Sambrook et al., 1989)

and the PCR product visualised and quantitated with a FujiBAS 1000 PhosphorImager. Levels of adenosine A_1 receptor transcripts were normalised to levels of β-actin transcript and expressed as a percent relative to the levels found in the kidneys of rats fed on a normal NaCl diet.

2.2.4. Autoradiography

On day 7, rats were anaesthetised with sodium thiopentone (120 mg kg^{-1}, i.p.) and a cannula placed in the abdominal aorta caudal to the left renal artery. A ligature was placed on the abdominal aorta above the right renal artery and both kidneys perfused retrogradely via the aortic cannula with sterile saline (0.9% w/v NaCl) for 1 min. The kidneys were rapidly removed, sliced longitudinally and immersed in 0.1 M sodium phosphate buffer, pH 7.4, containing 0.32 M sucrose for 2 min at 4°C. Kidneys were then frozen in isopentane cooled in liquid nitrogen. Tissue sections (20 μm) were cut at −18°C with a cryostat (Bright, UK), thaw mounted onto poly-*l*-lysine-coated slides and stored at −70°C until required.

Tissue sections were preincubated at 37°C in the presence of 1 u ml^{-1} adenosine deaminase (Brines and Forrest, 1988), 0.1 mM phenylmethylsulphonyl fluoride and 0.005% (v/v) polyethylenimine for 30 min, followed by a 4 h incubation at 4°C in the presence of 0.3 nM [^{3}H]DPCPX. Polyethylenimine was included to prevent adsorption of [^{3}H]DPCPX to glass slides. Nonspecific binding was determined by incubating consecutive tissue sections with 1 mM theophylline. Sections were washed twice for 15 min in ice-cold 50 mM Tris buffer, pH 7.4, rinsed in distilled water and dried in a stream of cold air. The sections were apposed to coverslips coated in LM1 nuclear emulsion (Amersham International, UK) and left to expose at 4°C for 12 weeks in light proof boxes. After exposure, the emulsion-coated coverslips were developed in Kodak-D19 (Kodak, UK) for 4 min at 18°C, rinsed for 30 s in distilled water, fixed for 5 min in Ilford Hy-pam (Ilford, UK) and finally rinsed in running water for 10 min. Consecutive sections were counterstained using haematoxylin and eosin.

Autoradiograms and counterstained sections were viewed under dark- and bright-field illumination, respectively, using a Leitz Dialux 20 microscope. Autoradiographic grain densities were quantified with a Leitz MPV compact microscope photometer. Images were captured using a Leica Quantimet 500+ image processing and analysis system. Ten readings of grain density were made in each kidney region for at least three sections per rat. Each experimental group comprised of four to five rats. The average background grain density was also determined and subtracted from sample values. Specific labelling of a particular kidney region was calculated by subtracting grain density for nonspecific binding from the corresponding value for total binding. Grain density was converted to the quantity of bound ligand by comparison with autoradiograms of ^{3}H standards (Amersham International). Calculations were made using the following formula (Geary and Wooten, 1983).

$$\text{fmol bound mg}^{-1}\text{ protein} = \frac{\mu\text{Ci bound}}{\text{specific activity (Ci mmol}^{-1})} \times \frac{10^6}{[\text{assay protein}]\,(\text{mg})}$$

Delineation of kidney regions was as described by The Renal Commission for the International Union of Physiological Sciences (IUPS) (1988). On the basis of results from autoradiographic studies, the inner medulla was divided into inner and outer zones of equivalent size.

2.2.5. Analysis of data

Data from binding experiments were analyzed by nonlinear least squares regression analysis (PCNONLIN, Statistical Consultants, 1986); and the goodness of fit, was determined statistically from an *F*-test on the sum of squares of the residuals. Estimates of K_d and B_{max} were obtained from the fitting procedures. Data are given as mean ± S.E. mean and statistical comparison of means was made using Student's unpaired *t*-test or, where appropriate, one way analysis of variance (ANOVA) with means compared by Dunnett's *t*-test. Differences were taken to be significant if $P < 0.05$.

3. Results

3.1. Effect of dietary NaCl on body weight, food and water intake, plasma Na^+ and urinary excretion

The results from rats used in binding experiments are given in Table 1. Similar data were obtained from animals used in RT-PCR and autoradiographic studies (data not

Table 1
Changes in body weight, food and water intake, plasma Na^+, urine output and urinary Na^+ excretion in rats maintained on a low (LS, 0.04%), normal (NS, 0.4%), or a high (HS, 4.0%) NaCl diet for 7 days
Values are given as mean ± S.E. mean with data derived from 12 rats.

	LS	NS	HS
Increase in body weight over 6 days (%)	17.2 ± 1.4[a]	20.0 ± 0.7	13.5 ± 1.2[b]
Daily food intake (g)[c]	25.3 ± 0.2[b]	35.4 ± 0.9	23.2 ± 0.8[b]
Daily water intake (ml)[c]	27.6 ± 0.6[b]	46.5 ± 1.7	91.7 ± 2.8[b]
Urine output (ml per 24 h)[d]	6.1 ± 0.7[b]	14.4 ± 3.3	84.7 ± 2.7[b]
Urine Na^+ (mmol per 24 h)[d]	0.5 ± 0.1[a]	3.3 ± 0.2	20.4 ± 1.5[b]
Plasma Na^+ (mM)[d]	148 ± 4	143 ± 2	145 ± 1

[a] $P < 0.05$ (ANOVA) relative to normal NaCl group.
[b] $P < 0.01$ (ANOVA) relative to normal NaCl group.
[c] Estimated for each rat from cages containing four rats with no corrections made for spillage.
[d] Urine collections and blood samples were taken on day 7.

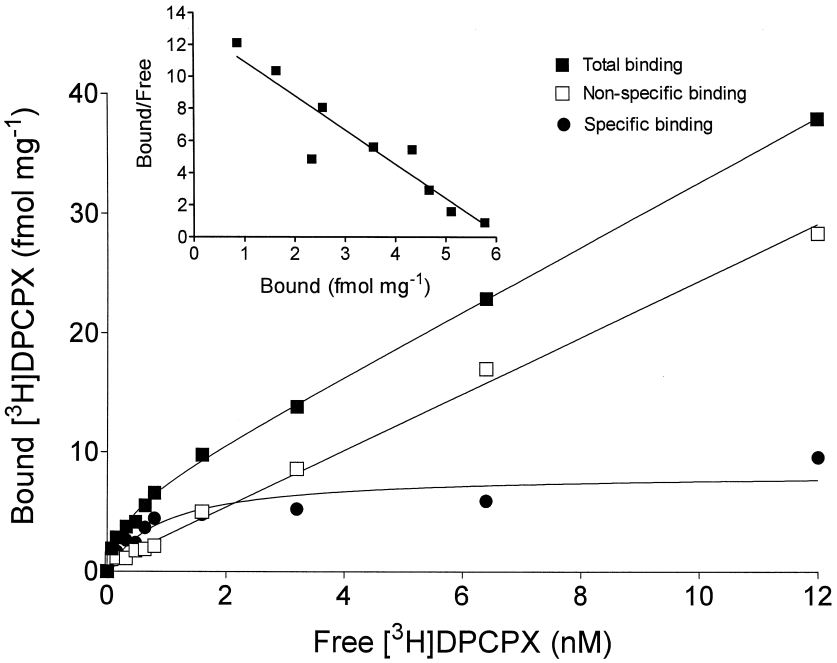

Fig. 1. Total, specific and non-specific binding of [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX) to renal cell membranes from rats fed for 7 days on a diet with a normal (0.4%) NaCl content. Points are means obtained from three experiments carried out with three to six replicates. Cell membranes were obtained from the kidneys of 24 rats. The insert shows a Scatchard plot produced from specific binding data.

shown). Both low and high NaCl diets resulted in reduced food intake and lower increases in body weight compared to animals given the normal diet. Water intake was also affected; a diet with a high NaCl content resulted in a twofold increase in daily water consumption whilst a 40% reduction in water intake occurred in rats on a low NaCl diet. Alterations in the salt content of diet had marked effects on urine output and Na^+ excretion although there were no changes in plasma Na^+ concentration. A low NaCl diet resulted in a 58% reduction in urine output

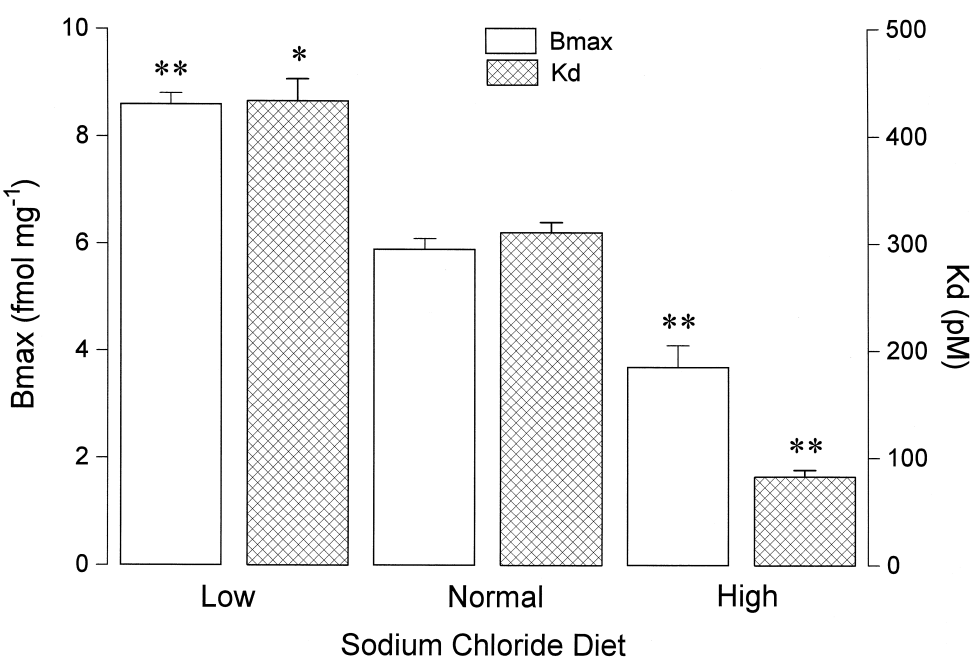

Fig. 2. Estimates of B_{max} and K_d for the binding of [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine to renal cell membranes from rats fed for 7 days on diets with a low (0.04%) ($n = 12$), normal (0.4%) ($n = 24$) or high (4.0%) ($n = 12$) NaCl content. Columns show mean values + S.E. mean. $^*P < 0.05$; $^{**}P < 0.01$ (Student's t-test) relative to values from rats on a normal NaCl diet.

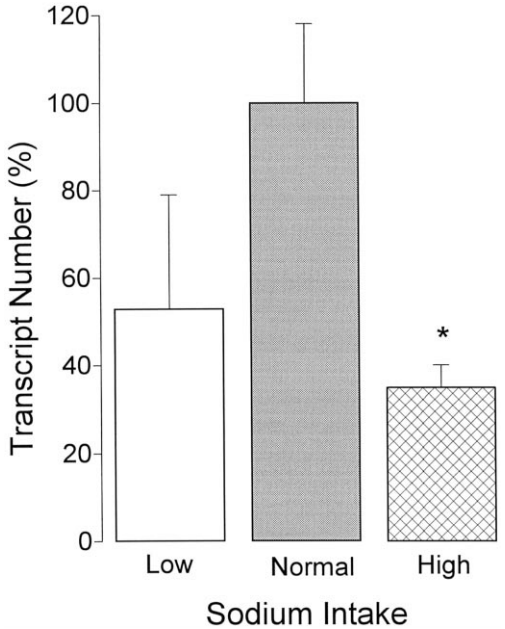

Fig. 3. Relative levels of adenosine A_1 receptor transcript in kidneys of rats fed for 7 days on diets with a low (0.04%), normal (0.4%) or high (4.0%) NaCl content. Levels of A_1 transcript were normalised to levels of transcript for β-actin and expressed relative to levels in kidneys of rats on a normal NaCl diet. Columns show mean values + S.E. mean. ($n = 5$) $^*P < 0.01$ (ANOVA) relative to values from rats on a normal NaCl diet.

accompanied by a marked decrease in urinary Na^+ excretion in comparison to rats on a normal salt diet. By contrast, a high NaCl diet significantly elevated urine output and urinary Na^+ excretion. The results in Table 1, and particularly data on urine flow and urinary Na^+ excretion, confirm that the modified diets had exposed kidneys of the respective groups of rats to low and high NaCl loads.

3.2. Binding of [^{3}H]DPCPX to renal cell membranes

Specific binding of [^{3}H]DPCPX to renal membranes was saturable and isotherms were best described by assuming interaction with a single class of homogenous binding sites. Binding to membranes of rats fed on a normal NaCl diet is shown in Fig. 1. The estimates of equilibrium dissociation constant, K_d, and density of binding sites, B_{max}, were 311 ± 9 pM and 5.9 ± 0.2 fmol mg^{-1} protein, respectively. These values are similar to those obtained previously (Gould et al., 1997). Analysis of saturation isotherms obtained from renal cell membranes of rats fed on low and high salt diets revealed that alteration of NaCl content of the diet was associated with changes in both K_d and B_{max} (Fig. 2). Increases of 39% and 46% in K_d and

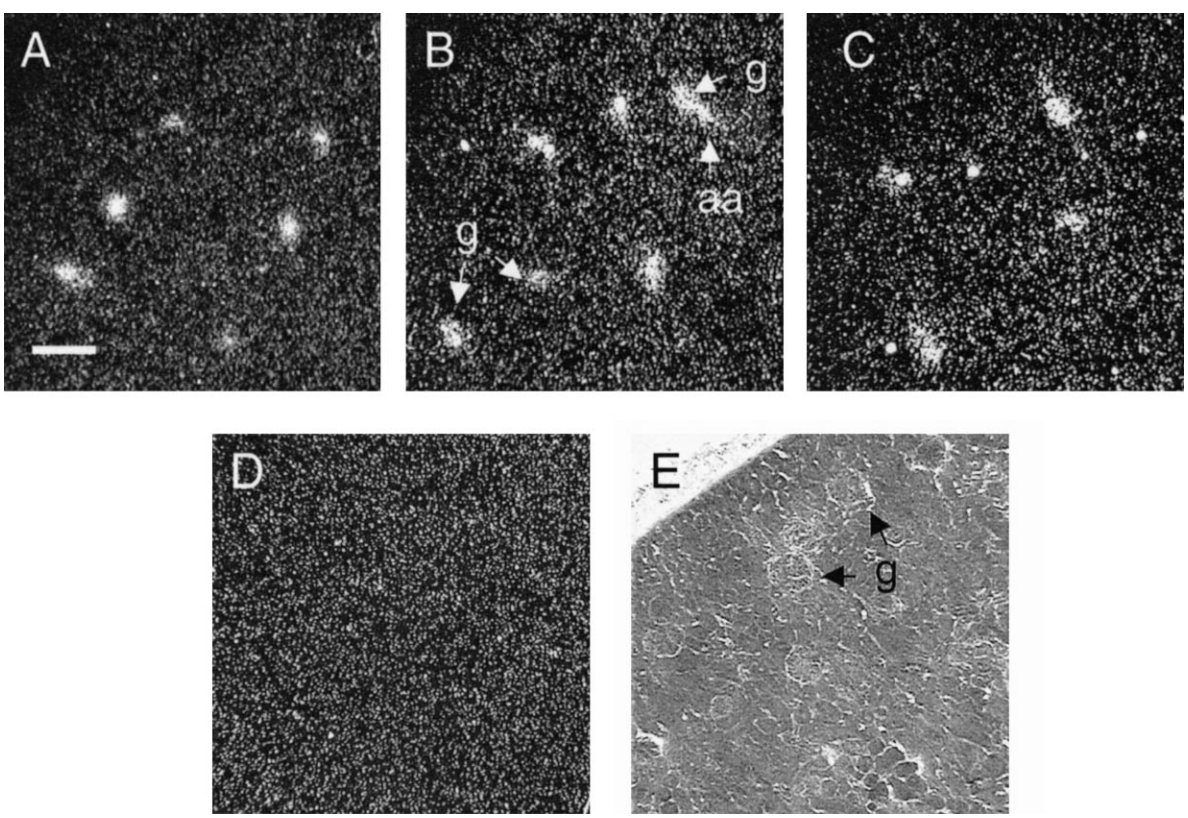

Fig. 4. Distribution of adenosine A_1 receptors in rat renal cortex. Panels (A), (B), (C) and (D) are autoradiograms showing the distribution of silver grains following incubation for 12 weeks with 0.3 nM [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX). Sections were taken from kidneys of rats fed for 7 days on diets with a low (0.04%) (A), normal (0.4%) (B), or high (4.0%) (C) NaCl content. Nonspecific binding was assessed by determining the binding of [^{3}H]DPCPX in the presence of 1 mM theophylline (D) whilst some consecutive sections were counterstained with haematoxylin and eosin (E). Key: g, glomeruli; aa, afferent arteriole. Scale bar in (A) represents 200 μm with all images taken at the same magnification.

B_{max}, respectively, were noted with cell membranes from rats on a low NaCl diet by comparison to rats given a normal salt diet. By contrast, a diet high in NaCl was associated with opposite changes in binding characteristics with decreases of 73% and 37% in K_d and B_{max}, respectively.

3.3. Adenosine A_1 receptor mRNA levels

The levels of adenosine A_1 receptor transcripts in the kidneys of rats fed a high NaCl diet were 65% ($P < 0.05$) lower than in rats given a normal NaCl diet (Fig. 3). By contrast, a low NaCl diet did not evoke any statistically significant change in receptor transcript levels.

3.4. Autoradiography

3.4.1. Binding of [^{3}H]DPCPX to sections of kidneys from rats fed a normal NaCl diet

In the cortical labyrinth, silver grains were concentrated in patchy circle-like structures that corresponded in location and diameter (60 to 90 μm) to glomeruli in consecutive stained sections (Fig. 4B and E). In a number of sections, the glomerulus was associated with a 'tail' of silver grains that might represent labelling of the afferent arteriole (Fig. 4B). No other structures were specifically labelled with [^{3}H]DPCPX in the cortical labyrinth or medullary rays. Longitudinal tubular structures were specifically labelled throughout the medulla with the exception of the outer stripe of the outer medulla. A typical autoradiogram and a corresponding stained section from the inner region of the inner medulla are shown in Fig. 5B and E. The density of binding of [^{3}H]DPCPX was calculated from grain densities and a summary of binding densities noted in the various regions of the kidney is shown in Fig. 6. The highest binding densities were in glomeruli and inner region of the inner medulla with virtually no labelling in the outer stripe of the outer medulla. Binding density in the inner stripe of the outer medulla and outer region of the inner medulla was about 40% of that in the glomeruli. The binding density in the inner region of the inner medulla was about twofold greater than in the adjacent outer region of the inner medulla.

3.4.2. Effect of changes in dietary NaCl on binding of [^{3}H]DPCPX to kidney sections

Changes in NaCl content of the diet were associated with significant changes in the density of adenosine A_1 receptors. Statistically significant alterations occurred in both areas of the inner medulla (Fig. 6); a low salt diet produced an increase in binding density whilst a high NaCl

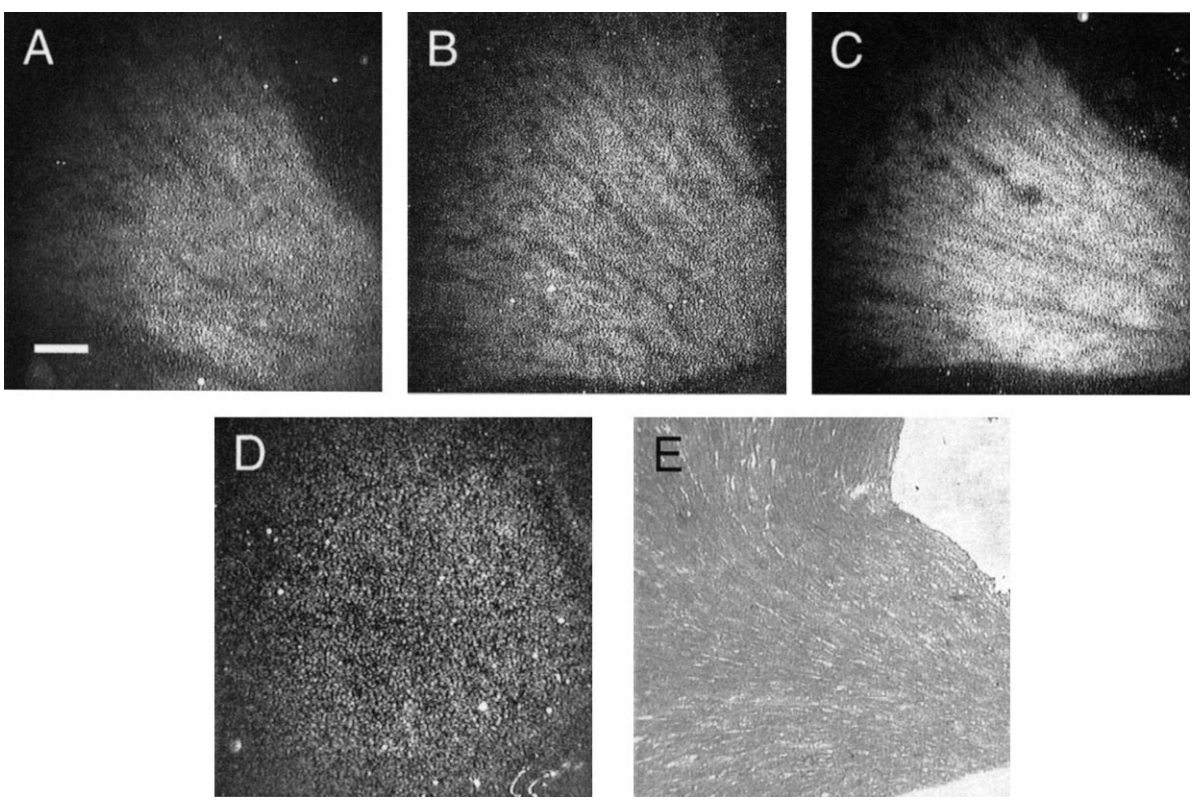

Fig. 5. Distribution of adenosine A_1 receptors in rat inner renal medulla. Panels (A), (B), (C) and (D) are autoradiograms showing the distribution of silver grains following incubation for 12 weeks with 0.3 nM [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX). Sections were taken from kidneys of rats fed for 7 days on diets with either a high (4.0%) (A), normal (0.4%) (B), or low (0.04%) (C) NaCl content. Nonspecific binding was assessed by determining the binding of [^{3}H]DPCPX in the presence of 1 mM theophylline (D) whilst some consecutive sections were counterstained with haematoxylin and eosin (E). Scale bar in (A) represents 500 μm with all images taken at the same magnification.

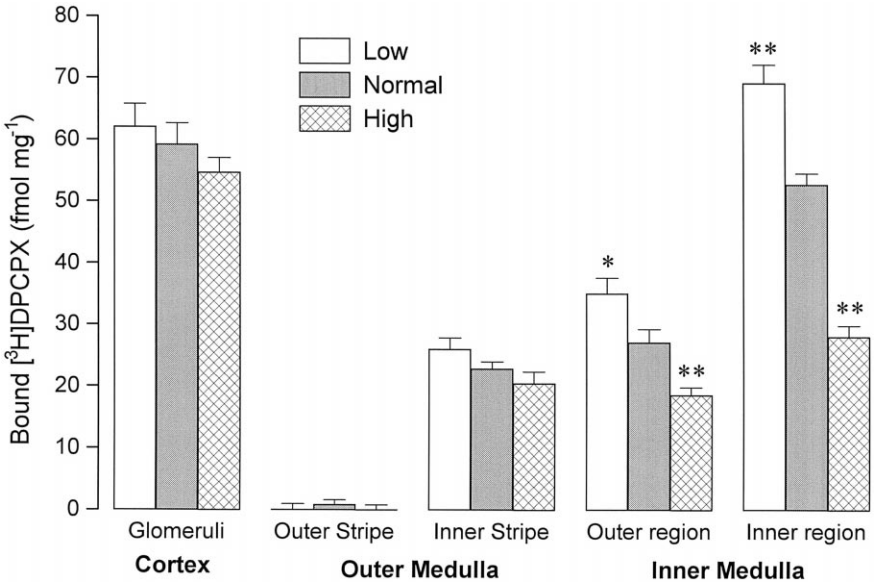

Fig. 6. Binding density of [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX) to various regions of the kidneys from rats fed for 7 days on diets with either a low (0.04%), normal (0.4%) or high (4.0%) NaCl content. Binding density was determined in 20 μm sections of the kidney. Ten readings of grain density (subsequently converted to binding density) were made in each kidney region for at least three sections per rat with four rats per dietary group. Columns show mean values + S.E. mean (n = 4–5). *$P < 0.05$; **$P < 0.01$ relative to the normal NaCl group (ANOVA).

diet resulted in a decrease in binding density. The magnitude of changes in binding density in the inner medulla (increases of 30% and decreases of 39%) was comparable to the changes in B_{max} determined from binding studies with renal cell membranes from rats given high and low salt diets (see Fig. 2). Typical autoradiograms of the inner region of the inner medulla, illustrating the changes in receptor density in response to alterations in dietary NaCl, are shown in Fig. 5A–C. By contrast to the changes in binding density noted in the medulla, alteration of the NaCl content of diet resulted in no changes in receptor density in glomeruli (Figs. 4A–C and 6).

4. Discussion

This study provides evidence that the binding characteristics of renal adenosine A_1 receptors are modulated by changes in NaCl intake. B_{max} for the binding of [^{3}H]DPCPX to renal membranes was decreased when rats were fed a diet containing a high salt content. This suggests down regulation of adenosine A_1 receptors in response to a high salt diet and this is supported by Zou et al. (1999) who, using Western blots, detected decreased levels of receptor protein in the cortex and medulla of rats fed a 4% salt diet for 3 weeks. In addition, our RT-PCR studies showed that the decrease in B_{max} is due to diminished receptor gene expression. By contrast to rats fed on a high salt diet, an increase in B_{max} was found for membranes from animals given a low NaCl diet. However, no increase in receptor mRNA could be detected by RT-PCR; hence the increase in B_{max} may result from enhanced translation and/or reduced receptor internalisation.

The changes in B_{max} may be a regulatory response to alterations in intrarenal adenosine levels. Siragy and Linden (1996) found a 14-fold increase in NaCl content of food fed to rats resulted in a 6.6-fold elevation of adenosine levels in the renal cortex and medulla. Conversely, a 46% decrease in the salt content of the diet lowered interstitial concentrations of adenosine in the renal cortex and medulla by a factor of 2.8 (Siragy and Linden, 1996).

Autoradiograms from rats on a normal salt diet showed that adenosine A_1 receptors are located in glomeruli, and tubular structures in the medulla. These observations support the findings of Palacios et al. (1987) who used the selective adenosine A_1 receptor agonist [^{3}H]cyclohexyladenosine as the ligand in autoradiography of human and guinea-pig kidneys. The ‘tail’ attached to glomeruli in some autoradiograms is most likely the afferent arteriole since there is functional evidence for the presence of adenosine A_1 receptors in this resistance vessel (Holz and Steinhausen, 1987), although adenosine A_1 receptors have not been visualized in this arteriole before. Weaver and Reppert (1992) studied adenosine receptor gene expression in rat kidney and, using in situ hybridization probes, detected A_1 mRNA in the juxtaglomerular apparatus only and not in the glomerulus. The circular pattern of grain density noted in the cortex in the current study and the diameter of the labelling pattern indicates that the receptor protein is present in the glomerular tuft as well as the juxtaglomerular apparatus. Adenosine A_1 receptors localized in the glomerular tuft may be present on mesangial

cells since, in the rat, adenosine A_1 receptors have been shown to produce contraction of these cells (Olivera et al., 1989). The most likely candidates for the labelled structures in the medulla are the collecting ducts since in situ hybridization studies showed that adenosine A_1 receptor mRNA in the rat kidney was most abundant in these tubular elements (Weaver and Reppert, 1992). The in situ hybridization studies of Weaver and Reppert (1992) noted that the medullary collecting ducts with the highest grain density were those in the papillary region. Similar observations were made in the current study, confirming that expression of adenosine A_1 receptor protein is greater in this area of the medullary collecting ducts. However, in addition to the collecting ducts, other structures in the medulla may also have been labelled since RT-PCR assays have detected adenosine A_1 receptor mRNA in the medullary thick ascending limb (Yamaguchi et al., 1995) and outer medullary descending vasa recta (Kreisberg et al., 1997). Moreover, binding studies in the rat have detected receptor protein in the medullary thick ascending limb, albeit at a density 20 times lower than in papillary membranes (Weber et al., 1990).

Adenosine A_1 receptors were not detected in the tubular elements in the cortex and the outer stripe of the medulla. These observations support the autoradiographic studies of Palacios et al. (1987) and Weaver and Reppert (1992) who visualised mRNA for the receptor. However, adenosine A_1 receptor mRNA has been detected in the proximal tubule, cortical collecting duct and cortical thick ascending limb of the rat using RT-PCR (Yamaguchi et al., 1995). The failure to detect adenosine A_1 receptor protein in the proximal tubule of cortical or juxtamedullary nephrons is surprising in view of the evidence that suggests a proximal tubule site of action of adenosine. Adenosine has been shown to stimulate sodium-coupled phosphate transport in the cultured opossum kidney epithelial cells, an immortalised cell line which has similar transport properties to the human proximal tubule cells (Coulson et al., 1991). Studies of rabbit proximal tubules indicate that endogenous adenosine, via an adenosine A_1 receptor, stimulates basolateral Na^+–HCO_3^- cotransport (Takeda et al., 1993). Moreover, studies in both rat and man suggest that the diuresis produced by selective adenosine A_1 receptor antagonists such as DPCPX and FK-453 ((+)-(*R*)-[(*E*)-3-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)acryloy]-2-piperidine ethanol) is primarily evoked by an action on the proximal tubule, with some activity at more distal nephron segments (Balakrishnan et al., 1993; Knight et al., 1993). The failure to detect A_1 receptors in the proximal tubule and a proximal site of diuretic action of adenosine antagonists remains to be reconciled.

In addition to changes in B_{max}, alteration of dietary salt appeared to modulate adenosine A_1 receptor affinity. An increase in K_d was found for membranes from rats on a low salt diet, whereas K_d was decreased for the high salt group. These changes in apparent affinity are the reciprocal of those noted for B_{max}, but the magnitudes of the alterations in K_d and B_{max} are similar. Thus it might be anticipated that there should be no change in the binding of [^{3}H]DPCPX to tissue sections from rats fed high or low salt diets. However, autoradiography showed that a high salt diet produced a decrease binding in the inner medulla whilst a low salt diet resulted in enhanced binding. Such changes in binding indicate downregulation and upregulation of adenosine A_1 receptors. However, in light of possible changes in adenosine A_1 receptor affinity in tissue slices, autoradiographic data should be interpreted with caution although, as discussed previously, the results from RT-PCR studies and the findings of Zou et al. (1999) provide convincing evidence for changes in receptor density.

By contrast to the present findings, the study of Zou et al. (1999) recorded a decrease in receptor density in the cortex as well as the medulla in response to a high NaCl diet, although this study did not further localise the reduction in receptor density. As discussed above, the most likely tubule structure for labelling in the inner medulla is the inner medullary collecting ducts. Our findings indicate that an increase in dietary Na^+ results in downregulation of adenosine A_1 receptors on the inner medullary collecting duct whilst a low salt diet produces receptor upregulation. Changes in dietary Na^+ produce alterations in interstitial concentrations of adenosine in both cortex and medulla (Siragy and Linden 1996), but the present study only detected changes in binding in one area of the medulla, that is, inner medullary collecting ducts. This suggests that changes in binding and therefore receptor regulation are not simply a response to altered adenosine levels.

Stimulation of adenosine A_1 receptors produces a twofold increase in number of channels $\times$ opening probability (NP_o) of Na^+ channels in A6 distal nephron cells (Ma and Ling, 1996). If adenosine A_1 receptors mediate a similar effect in the medullary collecting ducts, downregulation of these receptors will result in enhanced Na^+ excretion. Thus, a reduction in adenosine receptor density in the inner medullary collecting duct would help to remove an increased Na^+ load in animals given a high salt diet. The converse effect on renal function, that is increased Na^+ reabsorption, would result from upregulation of adenosine A_1 receptors in response to a diet low in NaCl.

In conclusion, we have shown that alteration in dietary NaCl produces changes in the density of adenosine A_1 receptors in medullary collecting ducts; increased salt intake produces a reduction in receptor density and a reduced salt intake results in increased density. These findings suggest that adenosine has, in addition to any short-term effects on renal function, a longer-term regulatory role in the control of tubular function. Such changes may play a role in the kidney's adaptive responses to alterations in NaCl intake and thereby contribute to blood and extracellular volume homeostasis.

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