ELSEVIER

PII S0024-3205(99)00549-4

Life Sciences, Vol. 65, No. 26, pp. 2801-2813, 1999

0024-3205/99/$–see front matter

CYCLOSPORINE A AND PURININERGIC RECEPTORS IN RAT KIDNEY

G. Halimi[1], J. Sampol[3], O. Clot-Faybesse[1], L. Mercier[1], C. Devaux[1], Y. Berland [2,3], B. Dussol[2,3], H. Rochat[1] and R. Guieu[1]

1 UMR CNRS 6560 Laboratoire de Biochimie et d'Ingenierie des Protéines. Faculté de Médecine Secteur Nord, Bd P Dramard Marseille France.
2 Centre d'Investigation Clinique 300 Bd Sainte Marguerite 13009, Marseille France
3 Service de Néphrologie, Hôpital Sainte Marguerite, 13009 Marseille, France.

(Received in final form August 13, 1999)

Summary

Previous reports have demonstrated that Cyclosporine A (CyA) chronically administered induces an increase in adenosine plasma concentration by inhibiting adenosine uptake by red blood cells (RBC). We hypothesized that this effect may modulate, by a down regulation, the m RNA expression of adenosine receptors in rat kidney. Since high blood pressure (HBP) is a classical side effect of CyA treatment, nicardipine, a dihydropyridine calcium channel blocker, is often associated with CyA in treatment. To distinguish between the effects of CyA-induced HBP and the effects of CyA by itself, we have evaluated the effects of CyA and/or nicardipine on the mRNA expression of A1 and A2a adenosine receptors. The study was performed on five groups of rats (n= 8) receiving during 21 days either serum saline (0.5 ml i.p), CyA (12mg/kg/day , i.p), nicardipine (1.2 mg/kg i.p) or nicardipine + CyA. The last (or fifth) group was injected with vehicle (0.5ml i.p). Blood samples for adenosine assay were collected in the renal artery at day 21, just before the rat kidneys were removed for quantitation of adenosine A1 and A2a mRNA concentration by RT-PCR. We make two conclusions :i) Nicardipine induces a decrease in mRNA expression of A1 but not of A2a adenosine receptors. However, because nicardipine lowered both blood pressure and A1mRNA expression, it is not possible to conclude if A1 mRNA decrease is implicated in the nicardipine effects on blood pressure.ii) CyA induces an increase in renal artery adenosine concentration and a decrease in mRNA expression of A1 and A2a adenosine receptors.

Key Words: **adenosine, cyclosporine, nicardipine**

Because of its immunosuppressive effect (1-3), Cyclosporine A (CyA) has marked beneficial effects in organ transplantation. However, hypertension and nephrotoxicity are two major side effects that seem related to the vascular toxicity of CyA (4-6). It has been suggested that vasoactive humoral substances such as angiotensin II (6) prostaglandin (7-8), and endothelin (9-10) mediate the renal vascular effects. Recently, it was shown that CyA inhibits adenosine (ADO) uptake by red blood cells (RBC), leading to an increase in ADO plasma concentration in kidney transplant recipients (KTR) (11). Adenosine acts on renal vascular tone via purinergic P1 receptors. Three subtypes of adenosine receptors have been identified in the kidney: A1 and A2a, which are abundant (12-14) and A2 b, which is present but poorly expressed (14). Renal vasoconstriction is mediated by the adenosine A1 receptor subtype (15) whereas vasodilatation is produced by stimulation of the A2a subtype (14 and for review see 15). The increase in ADO plasma concentration in renal vascular territorries may participate in CyA-induced high blood pressure (HBP) for two reasons: i) The acute administration of ADO into the renal artery increases systemic blood pressure, and the coadministration of adenosine deaminase which degrades adenosine into inosine, reverses this increase (16). ii) The renal toxicity of CyA is decreased by an adenosine antagonist, pentoxyphillin (17-18).

CyA can increase bradykinin B2 receptor number in the rat (19) and modulate the mRNA expression of endothelin A in kidney transplant recipients (20). Moreover, other immunosuppressive agents like glucocorticoids can regulate the expression of adenosine receptors in the nervous sytem (21). But to our knowledge, nothing is known about the effect of CyA on the mRNA expression of adenosine receptors in the kidney.

The aim of this study was to evaluate the mRNA expression of P1 purinergic receptors in rats chronically administered CyA. We also investigated the effects of nicardipine on the mRNA expression of P1 receptors. Indeed, calcium channel blockers are effective against CyA-induced nephrotoxicity (22).

Materials and Methods

Animals and treatments

Studies were performed on five groups of eight Sprague female rats (250-300g) bred in our laboratory. Grouping was as follows:

-group 1: serum saline 0.9% intra peritoneally (i.p) O.5 ml

-group 2: Cremophor ® (BASF Munich, Germany) 0.5 ml, i.p+ 0.5% methyl cellulose oral gavage.

-group 3: Cyclosporine A (Sandimmun®, Sandoz Laboratories, Rueil Malmaison France) 12 mg/kg/day i.p

-group 4: nicardipine (Loxen® Sandoz Laboratories, Rueil Malmaison France)) 1.2 mg/kg/day, i.p

-group 5: Cyclosporine A 12 mg/kg/day + nicardipine 1.2 mg/kg/day, i.p.

Animals were housed four per cage and were kept at a constant temperature (26°C). They had free access to food and water and were subjected to 12h cycles of light and dark. Cyclosporine, cremophor®® and serum saline were injected once a day between 9 and 11 am. Nicardipine was injected twice a day : 1 h before CyA injection in the morning and between 6 and 7 p.m. All the injections were performed during 21 days. Systolic blood pressure was measured with a tail blood pressure cuff, a pulse sensor attached to a piezoelectric crystal, and an automated electrophygmomanometer (Omron healthcare® Gmbh, Hamburg, Germany). The value recorded was the mean value of four measurements. For this procedure, the animals were lightly sedated with midazolam (Hypnovel® Roche Laboratory, Neuilly sur Seine, France) 0.15 mg/kg, i.p. Systolic blood pressure was evaluated two days before the administration of drugs, serum saline, or vehicle (cremophor®) and then at day 7 and 21. Body weights were evaluated on days 1, 7, 14 and 21 to adjust the dosage.

Adenosine assay.

Adenosine (crystallized, 99% pure), adenosine deaminase (calf intestine, specific activity 200 IU/mg), and dipyramidole (5 mg/ml) were from Boehringer Mannheim (Paris, France). Inosine (99% pure), α, β–methylene-adenosine-5'-diphosphate and deoxycoformycin were from Lederle Laboratories (Paris, France) 9-erythro (2-hydroxy-3-nonyl) adenine was from Burroughs Welcome. Heparin (25 IU/ml) was from Sanofi Winthrop (Gentilly France). Methanol, other reagents and the reversed phase chromatography column (Merck LIChrospher C18, 250 x 4 mm) were obtained from Merck (Dramstadt, Germany).

Blood samples.

Blood samples were collected at day 21 before the kidneys were removed. Rats were anaesthetized with penthotal (120mg/kg). Whole blood (1 ml) was drawn from the renal artery after laparotomia, using a butterfly connected to a special sample collection system for ultra rapid mixing with the cold stopping solution. With this method (see 23-24), the blood sample can be rapidly mixed with 3 ml of cold (0°C) stopping solution, which prevents ADO uptake by RBC (25-27). We checked that the mixed solution was correct by measuring hematocrits (mean values 46 ± 11 %; mean values of the hematocrits of blood plus stopping solution 17 ± 5 %). The stopping solution was composed of 0.2 mM dipyridamole, 4.2 mM Na_2EDTA, 5 mM EHNA, 79 mM AOPCP, heparin sulfate 1 IU/ml, and 0.9% NaCl. The sample plus stopping solution was centrifuged at 2500 g for 10 min and the supernatant was deproteinized by adding 70% perchloric acid v/v followed by a second centrifugation (2,500 g for 10 min). The supernatant was lyophilized and redissolved in 1 ml of 50 mM sodium phosphate buffer (pH 4). The resulting solutions were filtered by centrifugation in a Millipore Ultrafree-MC 0.45 µm filter before being chromatographed. Finally, 1ml of wole blood was collected from the renal artery for CyA peak levels.

HPLC.

Samples were analyzed by HPLC (Kratos HPLC 4000) with a 1 ml loop. Absorbance was measured at 254 nm, and eluted peak areas were measured with a Shimatzu Chromatopac C-RCA integrator. The technique has been described (23-24). Briefly, the column was equilibrated with 50 mM sodium phosphate buffer (pH 4). The sample was injected and was eluted with a methanol gradient (0 % to 46% methanol in 40 minutes) at a flow-rate of 1 ml/min. ADO was identified by elution time and by incubation with adenosine deaminase, which increases the inosine peak and decreases the ADO peak (see 23). ADO was quantified by comparison with the peak areas given by known quantities of adenosine. In these conditions, the sensitivity threshold was 10 pM injected in 1ml of plasma matrix.

Collection of tissues

Kidneys were rapidly dissected out after blood sample collection, snap frozen in liquid nitrogen, and stored at -70°C until use for subsequent sectioning and total RNA extraction.

RNA extraction

Total cellular RNA was extracted from tissue samples according to the acid-guanidine-thiocyanate-phenol-chloroform method of Chomczynski and Sacchi (28). The reagent was Trizol LS (Life Technologies, Gaithesburg, MD.).

Reverse transcription polymerase chain reaction (RT-PCR)

Six micrograms of total RNA was reverse transcribed in the presence of random hexamers by the use of M-MLV reverse transcriptase (Pharmacia Biotech Inc.) according to the manufacter's recommendations. Of the resulting cDNA, 2 µl was amplified by a PCR developed in our laboratory, with primers derived from the adenosine receptor cDNA sequences (Table 1). Beta-actin-specific primers sense 5'TTGTAACCAACTGGGACGATATGG 3' and antisense. 5'GATCTTGATCTTCATGGTGCTTAGG 3', designed to generate a 838 b p PCR product, served as internal standard. PCR was performed on a Perkin Elmer® thermocycler 9600 with 31 cycles at 94°C for 15 sec, 58°C for 30 sec and 72°C for 30 sec, and then a simple extension step of 72°C for 3 min. We followed the recommendations of Kwok and Higuchi (29) to avoid contamination. All RT-PCR reactions were performed on the same preparation for the analysis of the different mRNA receptors. After PCR a 10µl aliquot of each sample was electrophoresed on a 2% agarose gel, and the PCR product was visualized by ethidium bromide staining and a U V illuminator. PCR products were verified by sequence analysis.

Table 1: PCR primers and PCR product sizes of A1 and A2a adenosine receptors.

Receptor	Accession number	Primer	Nucleotides	Sequence	product sizes
A1	M64299	sense	295-315	5' CTGCCCATTGCTGTGGATCGA 3'	542 pb
		antisense	814-834	5' GTGTGTGAGGAAGATGGCGAT 3'	
A2a	L08102	sense	334-351	5' TTGGTGACAGGTGTGAGG 3'	225 pb
		antisense	536-556	5' GAAGGGGCAGTAACACGAACG 3'	

RT-PCR quantitation of adenosine A1 and A2a receptor mRNA concentrations

To compensate for variations in RNA isolation and tube-to-tube variations in RT and PCR reactions, multiplex RT-PCR can be performed by relative RT-PCR using β actin as an internal standard. Relative RT-PCR allows direct comparison between multiple samples. To increase reproducibility, the level of PCR product is adjusted, on the basis of the signal of the co-amplified internal standard. The PCR products were analyzed while the PCR was still in linear amplification for the target and the beta-actin. Quantitation was performed on the digital photography by an NIH image 6.1 software.

Statistical analysis

We used the Mann Whitney U-test to compare mRNA expression, adenosine concentration and systolic blood pressure and, the Wilcoxon test to compare systolic blood pressure as a function of time in the same group.

Results

CyA concentrations:

Whole blood CyA peak levels were 952±350 ng/ml in the 16 rats CyA administered.

Blood pressure (Figure 1)

Cremophor® did not modify systolic blood pressure relative to serum saline control group (mean+ SD 10.5±0.8 vs 9.9±1.2 cmHg). Rats under CyA treatment clearly develop systolic hypertension at day 21, since systolic blood press (mean ± SD = 14±2 cmHg) was higher than that of serum saline control group (mean+SD = 9.9±1.2 cmHg). On the contrary, rats under nicardipine alone developed systolic hypotension (mean + SD= 7.9±1 cmHg). Finally, at day 21, systolic blood pressure of rats under both CyA and nicardipine was in the same range at day 21 as serum saline controls (mean + SD= 10.2±1.2 vs 9.9±1.2 cmHg)

Adenosine plasma concentration (Table 2)

Adenosine plasma concentrations in CyA and CyA + nicardipine-treated rats were respectively 3.3 and 3-fold the values in rats administered serum saline (Table 2). Cremophor® or nicardipine alone did not modify ADO plasma concentration

Individual values, means and standard deviations (SD) of adenosine plasma concentrations evaluated in five groups of eight rats. Samples were collected in the kidney artery 21 days afyer drugs administration. We used a Mann Whitney U-test for statistical analysis. $p>.05$ was considered as significant.

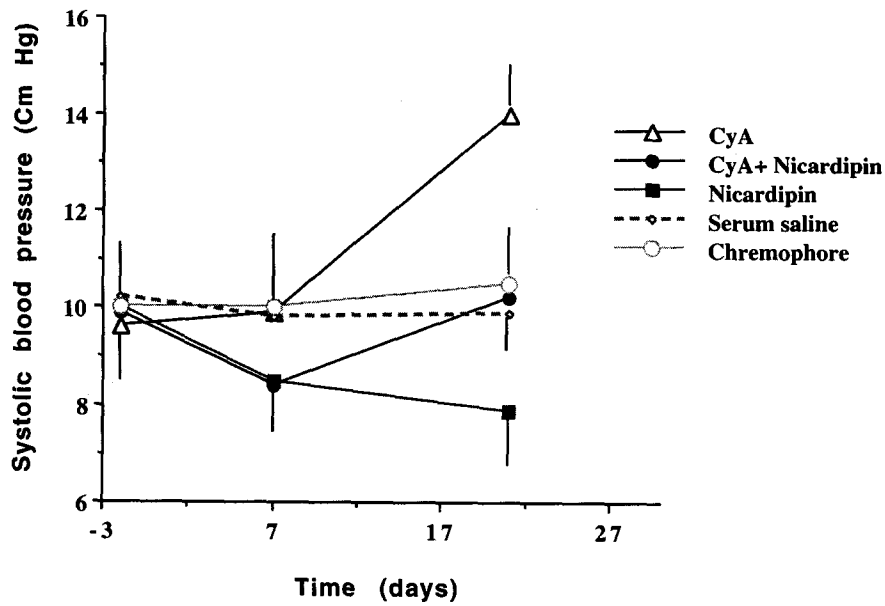


Fig. 1

Means and standard deviations of systolic blood pressure in five groups of eight rats during the administration of drugs, serum saline or vehicle (Cremophor®). The value recorded was the mean value of four measurements which were performed two days before the beginning of experimentation and at day 7 and 21. * Mann Whitney U-test $p<.05$ relative to serum saline control group. Δ Wilcoxon test, $p<.05$ relative to the first day of drug administration.

mRNA expression of adenosine receptors

Levels of adenosine A1 and A2a receptor mRNAs were expressed as the A1 recpetor/β-actin or A2a receptors/β–actin ratio of RT-PCR products (Figure 2). Cremophor® did not modify A1 or A2a m RNA receptor concentration relative to serum saline group (see Figure 3). CyA and /or nicardipine induced a significant decrease in mRNA A1 receptor concentration : mean of 58% for CyA alone, 61% for nicardipine alone, and 71% for the association CyA+ nicardipine, compared with controls (see Figure 3). CyA alone or coadministered with nicardipine induced a decrease in mRNA A2a receptor concentration that is in mean of 50 and 38 % respectively, relative to serum saline group, whereas nicardipine alone had no effect.

Table 2

p=0.0024

Groups	serum	saline	chremophor®	CyA	nicardipine	CyA+ nicardipine
	0.30	0.35	0.7	0.50	0.55
	0.35	0.50	1.4	0.50	1.2
	0.25	0.55	1	0.20	1
	0.45	0.35	0.9	0.20	1.3
	0.40	0.25	1.2	0.30	0.9
	0.20	0.28	1	0.40	0.8
	0.35	0.45	0.9	0.18	1
	0.19	0.25	1.2	0.19	0.8
Mean	0.31	0.37	1.03	0.3	0.94
SD	0.09	0.1	0.22	0.14	0.23

p=0.15;NS

p=0.37;NS

p=0.0027

Individual values, means, and standard deviations (SD) of adenosine plasma concentrations evaluated in five groups of eight rats. Samples were collected in the kidney artery 21 days after drug administration. We used a Mann Whitney U-test for statistical analysis. $P<.05$ was considered significant.

Discussion

In the rat kidney, nicardipine, a dihydropyridine calcium channel blocker, decreased the mRNA expression of A1 but not A2a adenosine receptor. The mechanism of this A1 "down regulation" is unclear. Interactions between adenosine and calcium channel blocker antagonists have been investigated in several tissues including cardiovascular system or central nervous system or even blood cells, but to our knowledge never in the kidney. Dihydropyridine inhibits nitrobenzylthioinisine binding to human erythrocyte nucleoside transporter (30-31), suggesting that these calcium channel blockers can inhibit ADO uptake by RBC and then increase ADO plasma concentration. However, we found that nicardipine does not modify ADO plasma concentration,

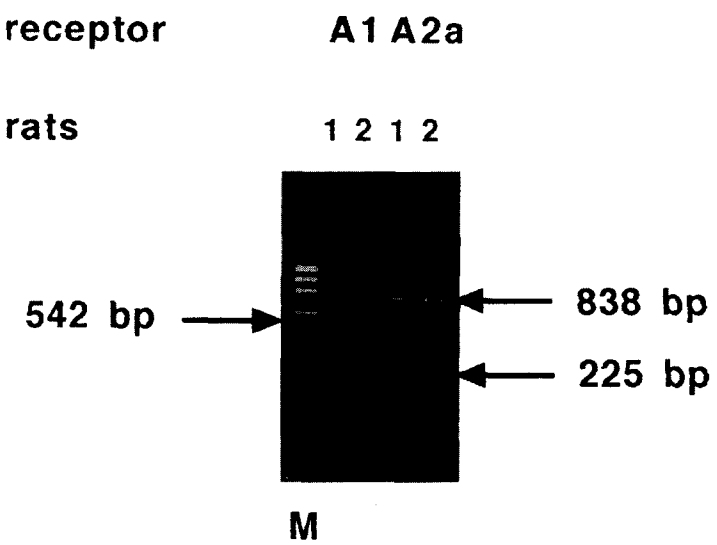
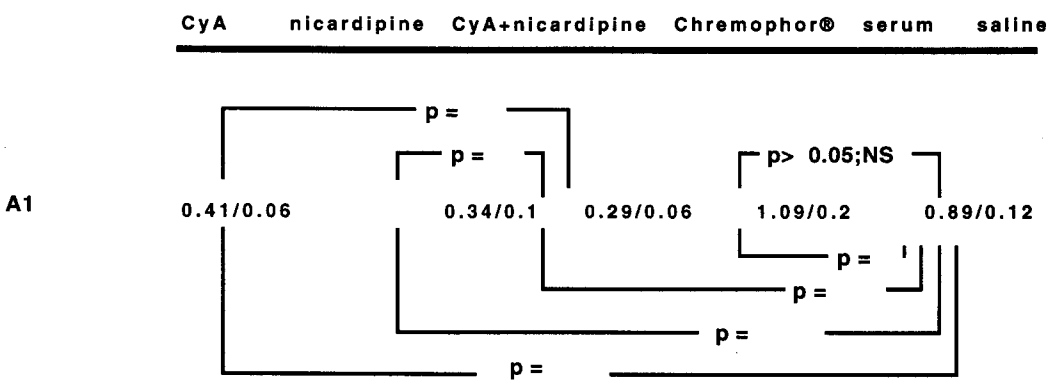


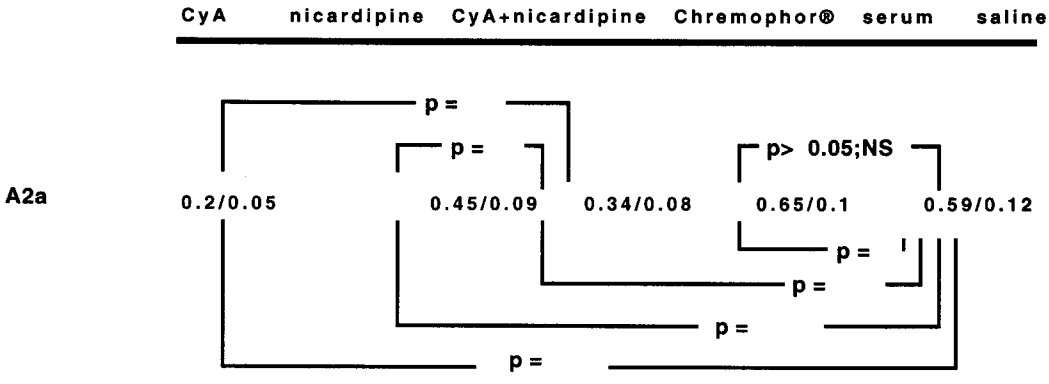
Fig. 2

2% agarose gel electrophoresis of RT-PCR products. The products size of ß actin, adenosine A1 and A2a receptors were respectively of 838 bp, 542 bp and 225 bp. Rats identified as 1 and 2, belonged to serum saline control group. M : molecular weight **size** marker phi-X 174 DNA digested with Hae III.

probably because the Ki values for dihydropyridine inhibition are in the higher micromolar range (31). Thus the decrease in mRNA expression of purinergic receptors is not secondary to the increase in ADO plasma concentration induced by nicardipine. Dihydropyridine inhibits the coronary vasodilatation action of adenosine in vivo (32) and interacts with adenosine receptors in guinea pig cerebral cortical preparations (33) and in rat cerebral cortex membranes (34).Nicardipine derivates bind to both A1 and A2a adenosine receptors in rat brain (35). Moreover, Hu *et al* .(36), reported no effect of dihydropyridine binding on A1 receptor-mediated adenylcyclase inhibition, suggesting that dihydropyridines may act as an antagonist of adenosine receptors in the central nervous system. They also reported that dihydropyridine derivates inhibit the binding of both agonists and antagonists of A1 adenosine receptors whereas non-dihydropyridine derivates have no effect. Some drugs can regulate the number of adenosine receptors in the cardiovascular or the nervous system. Thus chronic intra-cerebro-ventricular administration of morphine downregulates spinal adenosine A1 receptors in rats (37), whereas amiodarone downregulates A1 adenosine receptors in rat myocardial cells in vitro (38). Conversely, neuroleptics upregulates adenosine A2a receptors in rat striatum (39) whereas cisplatin upregulates adenosine receptors in the cochlea (40). However, in all cases, the precise mechanism of this drug-induced modulation of adenosine receptor number is unknown. Moreover, little is known about the effects of dihydropyridine derivates on kidney adenosine receptors.



A



B

Fig. 3

Means and standard deviations of adenosine A1 (Figure 3A) or A2a (Figure 3B) receptor mRNA concentrations in rat kidneys. Rats were injected intra-peritonealCy during 21 days with CyA (12mg/Kg/day) and/or nicarpidine (1.2mg/Kg/day) or vehicle (cremophor®; 0.5 ml/day). Those injected with serum saline (0.9%) served as controls.levels of adenosine A1 and A2a receptor mRNAs were expressed as the A1 or A2 receptor/β-actin ratio of RT-PCR products. We used the Mann Whitney U-test for statistical analysis. $p<.05$ was considered significant.

CyA chronically administered induced an increase in ADO plasma concentration and a decrease in the mRNA expression of both A1 and A2a adenosine receptors. CyA administration increases ADO plasma concentration by inhibiting ADO uptake by RBC (11). As a general rule, in the cardiovascular system, adenosine via the activation of A1 receptors decreases systemic blood pressure, by inhibiting noradrenergic neurotansmission (41). However, the kidney has a paradoxical response to adenosine. Indeed, acute adenosine infusion into the renal artery increases systemic blood pressure (16) by acting on the A1 receptors (15). We found high ADO concentration in the renal artery of rats chronically administered CyA which may explain the decrease in P1 receptor mRNA expression through a down regulation. This "down regulation" is probably not limited to the kidney; changes in mRNA expression could perhaps occur in the cardio-vascular system. However, extra renal modification of adenosine receptor function are probably poorly implicated since it is well established that the predominant effect of CyA induced HBP is renal vasoconstriction, mainly at preglomerular sites (42).This vasoconstriction may be mediated via enhanced vasoconstrictor prostaglandins, such as thromboxane (8), sympathetic nerve stimulation (43-44) and endothelin release (9-10). Another possible mechanism of CyA -induced renal vasoconstriction and then HBP is up regulation of A1 receptor or down regulation of A2a receptors (45). In our study, we found that CyA induced a decrease in both A1 and A2a mRNA expression. These decreases suggest that HBP induced by CyA treatment is not secondary to CyA-induced mRNA adenosine receptor decrease in the kidney and may also explain the lack of effect of A1 adenosine receptor antagonist. to prevent CyA-induced nephrotoxicity (46).

In conclusion, nicardipine chronically administered induces a decrease in A1 mRNA receptor concentration in rat kidney. However because nicardipine lowered both blood pressure and A1mRNA expression, it is not possible to conclude wether A1 mRNA decrease is implicated in the nicardipine effects on blood pressure. CyA chronically administered induces a decrease in both A1 and A2a mRNA adenosine receptor concentration. These results however, do not permit any conclusion on the modification of the receptor function.

References

1. F. BOREL, C. FEURER, H.U GUBLER and H STAEHLIN, Agents Actions **6** 468- 475 (1976).

2. R.Y CALNE, D.J WHITE, S. THIRU, D.B EVANS, P. McMASTER, D.C DUNN, G.N. CRADDOCK, B.D. PENTLOW, K. ROLLES, Lancet **2** 1323-1327 (1978).

3. D.J. COHEN, R. LOERTCHER, M.F. RUBIN, N.L. TILNEY, C.B. CARPENTIER, T.B. STROM, **101** 667-682 (1984).

4. J.L. CURTIS, R.G. LUKE, E. DUBOVSKY, A.G. DIETHELM, J.D. WHELCHEL, P. JONES, Lancet **2** 477-479 (1986).

5. F.J. KASKEL, P. DEVARAJAN, L.A ARBEIT, J.S PARTIN, L.C MOORE Am. J. Physiol. **252** F733-F742 (1987).

6 H. SIEGL B. RYFFEL Lancet **2** 1274 (1982).

7. C.R. BAXTER, G.G DUGGIN, J.S. HORVARTH, B.M. HALL, D.J. TIOLLER . Res. Commun. Chem Pathol. Pharmacol. **45** 69-80 (1984).

8. T.M. COFFMAN, D.R. CARR, W.E. YARGER, P.E. KLOTMAN Transplantation **43** 282-285 (1987).

9. N. PERICO, J. DADAN, G. REMUZZI. J. Am. Soc Nephrol. **1** 76-83 (1990)

10. Z.A. ABASSI, F. PIERNEZZI, F. NAKHBOUL, H.R. KEISER Hypertension **27** 1140-1148 (1996).

11. R. GUIEU, B. DUSSOL, C. DEVAUX, J. SAMPOL, P.BRUNET, H. ROCHAT and Y. BERLAND. Kidney Int. **53** 200-204 (1998).

12. M. FREISSMUTH, V. HAUSLEITHNER, E. TUISL,C. NANOFF, W. SCHUTZ Naunyn Schmiedberg 's Arch. Pharmacol. **335** 438-444 (1987).

13. M PALACIOS, J. FASTBOM, K.H. WIEDERHOLD, A. PROBST Eur J Pharmacol **138** 273-276 (1987).

14. D.R. WEAVER, S.M REPPERT. Am. J. Physiol. **263** F991-F995 (1992).

15. W.S. SPIELMAN, J AREND Hypertension **2** 117-130 (1991).

16. R.E KTHOLI, W.P. McCANN, W.T. WOODS. Hypertension **7** 188-193 (1985).

17. W.M. BENNET, L.W. ELZINGA, G.A. PORTIER, S. ROSEN,.Transplantation **54** 1118-1120 (1992).

18. M. CARRIER, L.P. PERRAULT, F.P. TRONC, D.J. STEWART, C.L. PELLETIER, Ann Thorac Surg **55** 490-492 (1993).

19. C. WANG, P. FORD, C. CHAO, L. CHAO, J. CHAO. Am J Physiol **273** F783-F789 (1997).

20. F.E. KARET, A.P. DAVENPORT. Nephrol. Dial. Transplant. **11** 1976-1982 (1996).

21 .P. SVENNINGSSON, B. FREDHOLM. J Pharmacol. Exp Ther **280** 1094-1101 (1997).

22. P. RUGGENETTI, N. PERICO, L. MOSCONI, F. GASPARI, A. BENIGNI, C.S. AMUCHASTEGUI, I. BRUZZI, J. REMUZZI. Kidney Int **43** 706-7121 (1993).

23. .R. GUIEUI, F. PAGANELLI, i F. SAMPIERI, G. BECHIS, S. LEVY, H. ROCHAT. Clin Chim Acta **230** 63-68 (1994).

24 .R. GUIEU, J.C. PERAGUT, P. ROUSSEL, H. HASSANI, F. SAMPIERI, G. BECHIS, R.GOLA, H. ROCHAT. Pain **68** 1-4 (1996).

25. J. ONTYD, J. SCHRADER. J Chromatogr **307** 404-409 (1984).

26 R.E. KLABUNDE, D.G. ALTHOUSE. Life Sci **23** 2631-2641 (1981).

27. J.C. SHRYOCK, M.T. BOYKIN, J.A. HILL, L. BELARDINELLI. Am J Physiol **258** H1232-H1239 (1990).

28. P. CHOMOCZYNSKI, N. SACCHI. Anal Biochem.**162** 156-159 (1987).

29. S. KWOK, R. HIGUSHI.Nature **339** 237-238 (1989).

30. J. STRESSING, G. ZERNIG, H. GLOSSMAN Eur J Biochem. **150** 67-77 (1985).

31. J.R. MAMMOND, E.F. WILLIAMS, A.S. CLANACHAN.Can J Physiol Pharmac **63** 1302 (1985).

32. G. MERRIL, M. YOUNG, S. DORREL, L. KRIEGER. Eur J Pharmacol **81** 543-550 (1982).

33. S. PSYCHOYOS, M. BAX, C. ATKINS. Biochem. Pharmacol **35** 1639-1646 (1986).

34. W.T. CHEUNG, M.M SHI, J.D. JOUNG, C.M. LEE.Biochem Pharmacol **13** 2183-2186 (1987).

35. .A.M. VAN RHEE, J.L. JIANG, N. MELMAN, M.E. OLAH, G.L. STILES, K.A. JACOBSON. J Med Chem **39** 2980-2989 (1996).

36. P.S. NU, E. LINDGREN, K.A. JACOBSON, B.B. FREDHOLM. Pharmacol Toxicol. **61** 121-125 (1987).

37. .P.L. WAO,C.F. LIU, H.C. TSAI . Eur J Pharmacol **278** 233-237 (1995).

38. D. EL ANI, K.A JACOBSON, A. SHAINBERG. Biochem. Pharmacol **54** 583-587 (1997).

39. B. PARSONS , D.M TOGASAKI, S. KASSIR, S. PRZEDBORSKI. J Neurochem. **65** 2057-2064 (1995).

40. M.S. FORD, Z. NIE, C. WITHWORTH, L.P. RYBACK, V. RAMKUMAR. Hear Res **11** 143-152 (1997).

41. E.K. JACKSON. Am J Physiol **253** H909-H918 (1987).

42. R.G LUKE. A J.H **4** 468-471 (1991).

43. N.G. MOSS, S.L. POWELL, R.J. FALK. Proc. Nat Acad Sci **82** 8222-8226 (1985).

44. R. CARTEIER, F. DAGENAIS, C. HOLLEMAN, H CAMBRON, J. BULURAN. Am. Thorac Surg **58** 789-794 (1994).

45 J GOULG, MJ MORTON, A SIVAPRASSADARAO, CJ BOWNER, MS YATES . Brit J Pharmacol **120** 947-953 (1997).

46 S. BALAKRISHNAN, C.J. VON RUHLAND, D.F.R GRIFFITHS, G.A COLES, J.D. WILLIAMS. Brit J Pharmacol **117** 879-884 (1996).