Effects of Adenosine A_1-Agonist and -Antagonist on Urinary Volume and Na Excretion in IAP-Treated and Non-Treated Rats

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ABSTRACT—Effects of an adenosine A_1-receptor agonist and antagonist were determined in pertussis toxin (IAP)-treated and non-treated rats. (−)-N^6-(2-phenylisopropyl) adenosine, an adenosine A_1-agonist, reduced the urine volume and sodium excretion without decreasing the glomerular filtration rate at 0.1 mg/kg (p.o.) in both IAP-treated and non-treated rats. Diuretic effects of KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine) and 8-cyclopentyl-1,3-dipropylxanthine, adenosine A_1-receptor antagonists, were not affected by pretreatment with IAP. These results suggest that endogenous adenosine may induce antidiuretic effects by accelerating the reabsorption of water and sodium at tubular sites via an IAP-insensitive mechanism, and that the diuretic effects of the adenosine A_1-receptor antagonist may result from inhibiting this action of endogenous adenosine.

Keywords: KW-3902, Adenosine A_1-receptor antagonist, Diuretic effect

It is reported that activation of adenosine A_1-receptors produces an antidiuretic effect by decreasing the glomerular filtration rate (GFR), resulting from the constriction of the afferent arterioles and mesangial cells in the kidney (1-4). Most of the adenosine A_1-receptors are coupled to various effectors through GTP binding inhibitory proteins (G_i proteins), and most of the effects of adenosine A_1-agonists are abolished by treatment of animals or cells with pertussis toxin (IAP). For example, inhibition of renin secretion and cAMP accumulation in rat renal cortical slices (5) and increase of cytosolic free calcium in rabbit cortical collecting tubule cells induced by adenosine A_1-agonists (6) are abolished by IAP pretreatment of the animals or cells. However, we reported previously that the diuretic effect of KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine), a selective and potent adenosine A_1-receptor antagonist, persists even in IAP-treated rats (7).

In the present study, we determined whether the antidiuretic effects of (−)-N^6-(2-phenylisopropyl) adenosine (R-PIA), an adenosine A_1-agonist, are always accompanied with a decrease of creatinine clearance (C_{CRE}), an index of GFR. Furthermore, whether the antidiuretic effect of R-PIA is abolished by IAP pretreatment was also investigated. If the antidiuretic effect of endogenous adenosine is mediated via an IAP-sensitive mechanism, the diuretic effect of an adenosine A_1-receptor antagonist must disappear in IAP-treated rats. To test this hypothesis, the diuretic effects of KW-3902 and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), which is an established adenosine A_1-receptor antagonist (8-10), was also studied in IAP-treated rats.

Male Wistar rats (Shizuoka Laboratory Animal Center, Inc., Hamamatsu) were used in the present studies. They were kept at 22℃ and on a 12 hr light-dark cycle. They had free access to tap water and commercial chow. Some rats were injected with IAP (10 μg/kg, i.v.) 7 days prior to the experiment. This dose of IAP totally abolished the bradycardic response to 5′-*N*-ethylcarboxamidoadenosine via adenosine A_1-receptors (7). KW-3902 and DPCPX were synthesized at Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd. IAP and R-PIA were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA).

R-PIA (0.1 or 1 mg/kg, p.o.) was administered to IAP-treated rats and non-treated rats with or without co-administration of KW-3902 (0.1 mg/kg, p.o.), and urine was collected for 4 hr. KW-3902 antagonizes the 5′-*N*-ethylcarboxamidoadenosine-induced bradycardic response which is thought to be mediated via adenosine A_1-receptors (7). Soon after the urine collection, blood was collected from the abdominal aorta under ether anesthesia and centrifuged (3000 rpm, 10 min, 4℃) to

obtain the serum. R-PIA and KW-3902 were suspended in saline containing 0.5 mg/ml Tween 80 and orally administered to rats at a volume of 25 ml/kg. To the rats in the control groups, only the solvent was orally administered at the same volume. After urine volume was measured, the concentration of sodium was determined by flame photometry (775-A; Hitachi, Ltd., Tokyo), from which sodium excretion was calculated. Concentrations of creatinine (CRE) in the urine and serum were determined by an autoanalyzer (AU510; Olympus, Tokyo), from which CCRE was calculated.

Table 1 shows the diuretic effects of KW-3902 and antidiuretic effects of R-PIA in IAP-treated and non-treated rats. KW-3902 caused a significant diuresis and natriuresis with no change in CCRE in both IAP-treated and non-treated rats. In IAP non-treated rats, R-PIA at a low dose of 0.1 mg/kg (p.o.) induced significant decreases of urine volume and sodium excretion without any influence on CCRE. At a high dose of 1 mg/kg, the antidiuretic effect of R-PIA was accompanied with a decrease of CCRE. These results suggest that the adenosine A_1-agonist causes antidiuretic effects not only by the decrease of GFR resulting from the constriction of the afferent arterioles and mesangial cells (1–4) but also by the acceleration of reabsorption of water and sodium at tubular sites. Similar effects of R-PIA were also observed in IAP-treated rats, suggesting that the renal effect of the adenosine A_1-agonist are mediated via an IAP-insensitive mechanism. These effects of R-PIA were completely abolished by the co-administration of KW-3902.

KW-3902 (0.1 mg/kg, p.o.) or DPCPX (1 mg/kg, p.o.) was administered to IAP-treated rats and non-treated

Table 1. Antidiuretic effects of R-PIA and the influence of KW-3902 in IAP-treated and non-treated rats

		Urine volume (ml/kg/4 hr)	Na excretion (mEq/kg/4 hr)	CCRE (l/kg/4 hr)
IAP-nontreated	control	9.9±0.5	1.03±0.06	0.97±0.05
	KW-3902 0.1 mg/kg (p.o.)	23.5±0.9[a]	3.53±0.15[a]	0.88±0.06
	+ KW-3902 0.1mg/kg (p.o.)	5.7±0.4**	0.29±0.02**	0.97±0.09
	R-PIA 1 mg/kg (p.o.)	11.8±0.7[b]	1.87±0.13[b]	0.94±0.09
	+ KW-3902 0.1 mg/kg (p.o.)	3.6±0.4**	0.18±0.02**	0.05±0.01**
		10.2±0.9[c]	1.22±0.13[c]	0.86±0.12[c]
IAP-treated	control	9.2±0.6	0.97±0.06	0.92±0.06
	KW-3902 0.1 mg/kg (p.o.)	23.6±0.8[a]	3.55±0.12[a]	0.87±0.08
	R-PIA 0.1 mg/kg (p.o.)	5.9±0.5**	0.30±0.02**	0.99±0.09
	+ KW-3902 0.1 mg/kg (p.o.)	11.7±0.7[b]	1.84±0.14[b]	0.95±0.07
	R-PIA 1 mg/kg (p.o.)	3.8±0.3**	0.19±0.01**	0.05±0.01**
	+ KW-3902 0.1 mg/kg (p.o.)	10.5±1.1[c]	1.21±0.13[c]	0.90±0.12[c]

Values represent means±S.E. of 6 animals. **: $P<0.01$, when compared with the control value by analysis of varlance (ANOVA) followed by Dunnett's test or by the Kruskal Wallis test followed by Steel's test. [a]: $P<0.001$, when compared with the control value by Student's *t*-test. [b]: $P<0.001$, when compared with the value in the R-PIA (0.1 mg/kg)-treated group by Student's *t*-test. [c]: $P<0.001$, when compared with the value in the R-PIA (1 mg/kg)-treated group by Student's *t*-test.

Table 2. Diuretic effects of KW-3902 and DPCPX in IAP-treated and non-treated rats

		Urine volume (ml/kg/4 hr)	Na excretion (mEq/kg/4 hr)	K excretion (mEq/kg/4 hr)
IAP-nontreated	control	17.7±1.0	2.63±0.10	1.20±0.05
	KW-3902 0.1 mg/kg (p.o.)	33.0±1.2**	5.06±0.23**	1.34±0.06
	DPCPX 1 mg/kg (p.o.)	30.8±1.4**	4.91±0.20**	1.35±0.07
IAP-treated	control	15.9±1.5	2.60±0.22	1.02±0.03
	KW-3902 0.1 mg/kg (p.o.)	26.9±1.3**	4.82±0.11**	1.38±0.06
	DPCPX 1 mg/kg (p.o.)	26.3±1.9**	4.65±0.22**	1.26±0.07

Values represent means±S.E. of 8 animals. ** $P<0.01$, when compared with the control value by analysis of varlance (ANOVA) followed by Dunnett's test.

rats. Drugs were suspended in saline containing 0.5 mg/ml Tween 80, and the suspensions were orally administered to rats at a volume 25 ml/kg. Urine was collected for 4 hr, and the urine volume and concentrations of sodium and potassium were determined.

As shown in Table 2, the diuretic effect of KW-3902 was not abolished by IAP treatment and persisted to an extent similar to that in IAP non-treated rats. Furthermore, DPCPX, which is another adenosine A_1-receptor antagonist, induced diuretic effects in both IAP-treated rats and non-treated rats, like KW-3902. These results support the notion that the diuretic effect of the adenosine A_1-receptor antagonist is exhibited through an IAP-insensitive mechanism (7).

We reported previously that the diuretic effect of KW-3902 is produced by inhibiting reabsorption of water and sodium at tubular sites but not by affecting renal hemodynamics in normal rats (7, 11). The results from the previous study and the present one suggest that endogenous adenosine may induce antidiuretic effects by accelerating reabsorption of water and sodium at tubular sites via an IAP-insensitive mechanism, and that the diuretic effects of the adenosine A_1-receptor antagonist may result from inhibiting this action of endogenous adenosine.

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