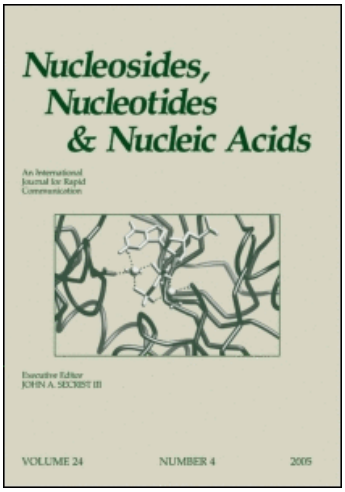


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Recognition of Adenosine Receptors by Amiloride and Its Analogues

A. Garritsen[a]; M. W. Beukers[a]; A. P. Ijzerman[a]; W. Soudijn[a]
[a] Center for Bio-Pharmaceutical Sciences, Dept. Medicinal Chemistry, RA Leiden, The Netherlands

To cite this Article Garritsen, A. , Beukers, M. W. , Ijzerman, A. P. and Soudijn, W.(1991) 'Recognition of Adenosine Receptors by Amiloride and Its Analogues', Nucleosides, Nucleotides and Nucleic Acids, 10: 5, 1107 — 1111
To link to this Article: DOI: 10.1080/07328319108047248
URL: http://dx.doi.org/10.1080/07328319108047248

NUCLEOSIDES & NUCLEOTIDES, 10(5), 1107-1111 (1991)

RECOGNITION OF ADENOSINE RECEPTORS BY AMILORIDE AND ITS ANALOGUES

A. Garritsen, M.W. Beukers, A.P. IJzerman and W. Soudijn*
Center for Bio-Pharmaceutical Sciences, Dept. Medicinal Chemistry
P.O. Box 9502, 2300 RA Leiden, The Netherlands

Abstract. Amiloride and its analogues displace the adenosine A_1 receptor ligands [^{3}H]CPDPX and [^{3}H]PIA from their binding sites in calf brain membranes in a GTP-insensitive manner. High [NaCl] or low pH reduces the affinity of amiloride for A_1 receptors, whereas the affinity of [^{3}H]CPDPX is not affected. Notwithstanding this difference in modulation, the interaction between amiloride and A_1 receptors appears competitive in nature. The structure-affinity relationships differ from those for classic amiloride-sensitive Na^+ transport systems, indicating that a coupling between the A_1 receptor and one of these systems is very unlikely. Amiloride and its analogues may represent a novel class of A_1 receptor antagonists.

INTRODUCTION

Amiloride, a potassium sparing diuretic, and its analogues have been used as tools in the investigation of several forms of Na^+ transport[1]. Amiloride analogues substituted at the 5-NH_2 position are potent inhibitors of the plasma membrane Na^+/H^+ exchanger, whereas substitution at a terminal guanidino nitrogen atom results in compounds that are virtually inactive at this exchanger, but potent inhibitors of the epithelial sodium channel[1]. Interestingly, in the 10-100 μM range the drugs also affect receptor binding, a.o. to adrenergic and muscarinergic receptors[2-4]. Combination of this finding with the knowledge that Na^+ plays a role in receptor-mediated inhibition of adenylate cyclase led to the intriguing hypothesis that the -effect of amiloride may reflect a direct link between G_i-coupled receptors and Na^+/H^+ exchange[5].

In the present paper, we show that amiloride and its analogues also inhibit radioligand binding to the (G_i-coupled) adenosine A_1 receptor. The characteristics of the interaction are explored and the structural requirements for displacement of [^{3}H]CPDPX binding are compared with those for the classic effects of amiloride and its analogues.

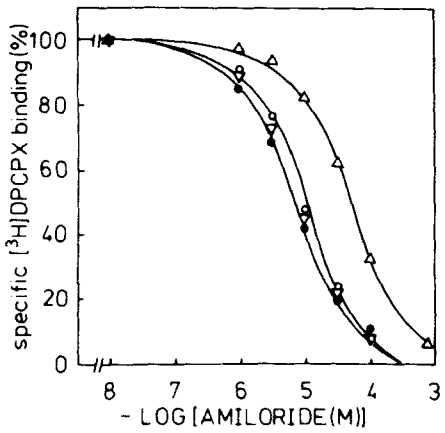


FIG. 1. Displacement of [^{3}H]CPDPX binding by amiloride: effect of GTP and salts. Calf brain membranes were incubated with [^{3}H]CPDPX for 1 h at 25°C in the absence (● ; K_i 2.0 ± 0.2 μM) or presence of 1 mM GTP (▽ ; K_i 3.0 ± 0.3 μM), 145 mM NaCl (△; K_i 20 ± 1 μM) or 145 mM choline chloride (○ ; K_i 4.5 ± 0.4 μM)

METHODS

Calf brain membranes were prepared according to Van Galen et al.[6]. Binding assays were performed and analyzed as described previously[7]. Amiloride was kindly donated by Merck Sharp & Dohme (Haarlem, The Netherlands), MBA by Dr. G. Schmalzing (Frankfurt, FRG) and other amiloride analogues by Dr. E.J. Cragoe (Lansdale, USA).

RESULTS AND DISCUSSION

Amiloride inhibits binding of the A_1 antagonist [^{3}H]CPDPX with an affinity in the low micromolar range (FIG. 1). The displacement curve is steep and not affected by GTP, suggesting an antagonist-like way of interaction. Interestingly, the affinity of amiloride is reduced by NaCl (FIG. 1) and by decreasing the pH of the medium (FIG. 2). Full displacement curves at pH 6.6, 7.3 and 8.4 result in K_i values for amiloride of 9.3±0.7, 3.4±0.2 and 1.3±0.1 μM, respectively. 145 mM NaCl does not affect the affinity of [^{3}H]CPDPX, although it increases the B_{max} by 25%. The latter effect is mimicked by choline chloride. [^{3}H]CPDPX binding is unaltered by the pH changes (FIG. 2). It should be noted that in all experiments virtually identical results were obtained with the A_1 agonist [^{3}H]PIA. This differential modulation suggests that amiloride interacts with a domain at the A_1 receptor distinct from that of the classic A_1 antagonists. This prompted us to further explore the nature of the interaction.

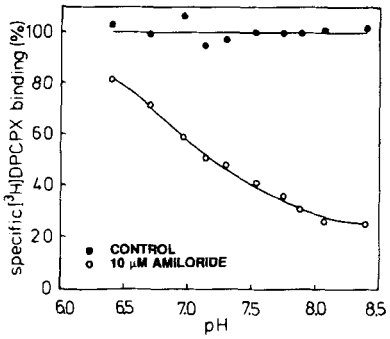


FIG. 2. Displacement of [³H]CPDPX binding by amiloride: effect of pH. Calf brain membranes were incubated with [³H]CPDPX for 1 h at 25°C at different pH values in the absence (●) or presence (○) of 10 μM amiloride.

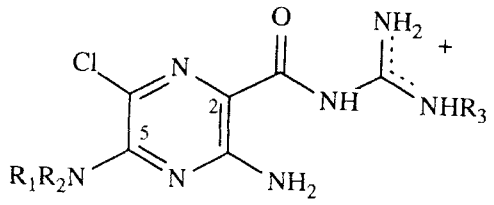
The binding parameters of [³H]CPDPX in calf brain membranes were determined in the presence of 2 μM amiloride, 70 nM MBA and 0.65 μM benzamil. Consistent with (allosteric) competition, the three compounds reduce the affinity of the radioligand from 72±3 to 139±6 (amiloride), 200±7 (MBA) and 150±5 pM (benzamil). The maximal binding capacity is virtually unchanged (808, 901 and 832 vs. 800 fmol/mg for the control).

To investigate whether the interaction is allosteric in nature, the dissociation rate of the [³H]CPDPX-receptor complex, induced by 8-phenyltheophylline, was measured in the presence of amiloride (20 μM), MBA (0.7 μM) and benzamil (6.5 μM). In these concentrations none of the drugs affected the dissociation rate constant (data not shown). This suggests that the interaction between amiloride and its analogues and the A_1 receptor, in contrast to the α_2-adrenoceptor[4], is not allosteric in nature.

Besides amiloride, several analogues were tested that are reportedly selective inhibitors of the epithelial sodium channel, Na^+/H^+ exchanger or Na^+/Ca^{2+} exchanger[1]. TABLE 1 shows that the 5-NH_2-dialkyl-substituted analogues are more effective in inhibiting [³H]CPDPX binding than amiloride. However, although the guanidino-substituted analogues are devoid of affinity for the Na^+/H^+ exchanger[1], they are more potent than amiloride in displacing [³H]CPDPX binding. From this structure-activity profile we conclude that none of the amiloride-sensitive Na^+ transport systems is involved and there is evidently no support for a link between the G_i-coupled A_1 receptor and Na^+/H^+ exchange, as postulated by Limbird[5].

Amiloride and its analogues may represent a novel class of adenosine receptor antagonists. The compounds, however, are not selective for A_1 receptors. In rat brain the affinities of amiloride, HMA and benzamil for A_2 receptors are similar to those for A_1 receptors. Furthermore, although adenosine receptors are among the most sensitive towards

TABLE 1. *Inhibition constants of amiloride and its analogues for displacement of [^{3}H]CPDPX and [^{3}H]PIA binding to calf brain membranes. Values were calculated from the combined data of 4-8 experiments.*



Drug	R_1	R_2	$K_i \pm$ SE (μM) [^{3}H]CPDPX		[^{3}H]PIA	
Na^+/H^+ exchange inhibitors: 5-amino-substituted derivatives (R_3=H)						
Amiloride	H	H	2.0	± 0.2	2.4	± 0.1
MGCMA	$(NH_2)_2C$=N-CO-CH_2-	CH_3	22	± 1	16	± 1
HMA	-$(CH_2)_6$-		0.41	± 0.03	0.50	± 0.03
MBA	$CH_3(CH_2)_3$-	CH_3	0.070	± 0.004	0.092	± 0.010
MIBA	$(CH_3)_2CHCH_2$-	CH_3	0.16	± 0.01	0.20	± 0.01
Na^+ channel inhibitors: 2-guanidino-substituted derivatives (R_1=R_2=H)						
	R_3					
Benzamil	$C_6H_5CH_2$-		0.65	± 0.04	0.85	± 0.03
Phenamil	C_6H_5-		1.5	± 0.1	2.3	± 0.1
DCB	*m,p*-$Cl_2C_6H_3CH_2$-		1.6	± 0.1	2.7	± 0.2
Na^+/Ca^{2+} inhibitor: CBDMB (R_1=H)						
R_2 =	*p*-$ClC_6H_4CH_2$-		1.2	± 0.1	4.0	± 0.4
R_3 =	*o,p*-$(CH_3)_2C_6H_3CH_2$-					

Abbreviations: CBDMB, 5-(N-4-chlorobenzyl)-2',4'-dimethylbenzamil; CPDPX, 8-cyclopentyl-1,3-dipropylxanthine; DCB, 3',4'-dichlorobenzamil; HMA, 5-(N,N-hexamethylene)amiloride; MBA, 5-(N-butyl-N-methyl)amiloride; MGCMA, 5-[N-(guanidinocarbonylmethyl)-N-methyl]amiloride; MIBA, 5-(N-isobutyl-N-methyl)amiloride; PIA, N^6-R-1-phenyl-2-propyladenosine.

amiloride and its analogues, many other receptors are affected as well and some of these (e.g. the μ-opiate receptor) are even more sensitive to the drugs (data not shown)[2,4]. The fact that amiloride and its analogues inhibit binding to many receptors suggests that they interact with a highly conserved domain, that is close enough to the ligand binding site to affect binding of a radioligand.

This class of compounds may lead to interesting adenosine receptor antagonists that are structurally unrelated to the xanthines and seem to bind to a domain at the A_1 receptor that differs from the binding domain of the classic ligands.

REFERENCES

1. Kleyman, T.R.; Cragoe, E.J. Jr. *J. Membrane Biol.* **1988**, *105*, 1-21.
2. Howard, M.J.; Mullen, M.D.; Insel, P.A. *Am. J. Physiol.* **1987**, *253*, F21-25.
3. Kuijpers, G.A.J.; De Pont J.J.H.H.M.; Van Nooy I.G.P.; Fleuren-Jakobs, A.A.M.; Bonting S.L.; Rodriguez de Miranda, J. *Biochim. Biophys. Acta*, **1984**, *804*, 237-244.
4. Nunnari, J.M.; Repaske, M.G.; Brandon, S.; Cragoe, E.J. Jr.; Limbird, L.E. *J. Biol. Chem.* **1987**, *262*, 12387-12392.
5. Limbird, L.E. *FASEB J.* **1988**, *2*, 2686-2695.
6. Van Galen, P.J.M.; IJzerman, A.P.; Soudijn, W. *FEBS Lett.* **1987**, *223* 197-201.
7. Garritsen, A.; IJzerman, A.P.; Beukers, M.W.; Cragoe, E.J. Jr.; Soudijn, W. *Biochem. Pharmacol.* **1990**, (in press)