Species differences in structure-activity relationships of adenosine agonists and xanthine antagonists at brain A1 adenosine receptors

Dieter Ukena, Kenneth A. Jacobson*, William L. Padgett, Cristina Ayala, Mah T. Shamim, Kenneth L. Kirk*, Ray O. Olsson† and John W. Daly

*Laboratory of Bioorganic Chemistry and *Laboratory of Chemistry, National Institute of Diabetes, and Digestive and Kidney diseases, National Institutes of Health, Bethesda, MD 20892* and †*Department of Internal Medicine, University of South Florida, College of Medicine, Tampa, FL 33612, USA*

Received 2 October 1986

A series of 28 adenosine analogs and 17 xanthines has been assessed as inhibitors of binding of N^6-*R*-[^{3}H]-phenylisopropyladenosine binding to A1 adenosine receptors in membranes from rat, calf, and guinea pig brain. Potencies of N^6-alkyl- and N^6-cycloalkyladenosines are similar in the different species. However, the presence of an aryl or heteroaryl moiety in the N^6 substituent results in marked species differences with certain such analogs being about 30-fold more potent at receptors in calf than in guinea pig brain. Potencies at receptors in rat brain are intermediate. Conversely, 2-chloroadenosine and 5′-*N*-ethylcarboxamidoadenosine are about 10-fold less potent at receptors in calf brain than in guinea pig brain. Potencies of xanthines, such as theophylline, caffeine and 1,3-dipropylxanthine are similar in the different species. However, the presence of an 8-phenyl or 8-cycloalkyl substituent results in marked species differences. For example, a xanthine amine conjugate of 1,3-dipropyl-8-phenylxanthine is 9-fold more potent at receptors in calf than in rat brain and 110-fold more potent in calf than in guinea pig brain. Such differences indicate that brain A1 adenosine receptors are not identical in recognition sites for either agonists or antagonists in different mammalian species.

Adenosine agonist *Xanthine antagonist* *Adenosine receptor* *Structure-activity*

1. INTRODUCTION

A wide range of studies have provided evidence for two classes of adenosine receptors, one inhibitory (A1) and the other stimulatory (A2) to adenylate cyclase (reviews [1,2]). In addition to having opposite effects on the adenylate cyclase activity, the two types of receptor differ according to the potency order within a limited set of adenosine analogs. However, ambiguity has arisen when these analogs were used to identify the type of receptor involved in physiological responses, either in vivo or in isolated tissues. This led to the suggestion that there is but one type of adenosine receptor [3]. One reason for ambiguous results may be species differences in adenosine receptors. Initial studies [4,5] showed differences in the potency of various ligands at binding sites in rat, calf and guinea pig brains: the binding properties of tritiated adenosine analogs were similar in brain of different species, while the antagonist 1,3-diethyl-8-phenylxanthine showed considerable differences in affinity for brain A1 receptors. Recent studies indicate that the [^{3}H]xanthine amine congener of the antagonist 1,3-dipropylxanthine also shows marked species differences in affinity for brain A1 receptors [6].

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

2. MATERIALS AND METHODS

Membranes from rat, calf and guinea pig cerebral cortex were prepared as described [6]. The binding of 1 nM ^{3}H-labeled N^6-*R*-1-phenyl-2-propyladenosine ([^{3}H]PIA) to these membranes at 37°C was assayed essentially as in [6]. IC_{50} values were transformed into K_i values using a K_d for [^{3}H]PIA binding of 1.0 nM in rat, 0.52 nM in calf and 4.9 nM in guinea pig brain [6]. Chemicals and materials were from standard sources [6].

Synthetic procedures have been described for the various adenosine analogs and xanthines or were by standard methods (see [7–12]).

3. RESULTS

N^6-Alkyl- and N^6-cycloalkyladenosine analogs (compounds **1–8**) show nearly the same affinity at

Table 1

Inhibition of [^{3}H]PIA binding to cerebral cortex membranes by adenosine analogs

Adenosine analog	Inhibition [^{3}H]PIA binding (K_i, nM)		
	Rat	Calf	Guinea pig
1 N^6-1-Butyl-	6.3 (4.4–9.1)	10.7 (9.2–13.4)	13.2 (9.5–18.3)
2 N^6-Cyclobutyl-	0.84 (0.59–1.2)	1.7 (1.3–2.3)	1.8 (1.2–2.6)
3 N^6-3-Pentyl-	1.0 (0.69–1.5)	1.6 (1.2–2.1)	2.1 (1.4–3.2)
4 N^6-2-Methyl-2-propyl-	20.4 (12.2–34)	57 (33–100)	42 (30–57)
5 N^6-Cyclopentyl-	0.34 (0.27–0.41)	0.69 (0.53–0.89)	1.1 (0.87–1.4)
6 N^6-1-Methylcyclopentyl-	1.4 (1.0–1.9)	6.7 (4.7–9.4)	5.1 (2.8–9.0)
7 N^6-Cyclohexyl-	1.0 (0.69–1.6)	1.4 (0.90–2.2)	2.4 (1.6–3.7)
8 N^6-Cyclooctyl-	5.4 (4.2–6.8)	5.4 (4.5–6.5)	8.1 (7.6–8.5)
9 N^6-Phenyl-	16.4 (9.7–28)	3.0 (1.6–5.7)	110 (67–180)
10 N^6-Benzyl-	175 (120–260)	59 (48–73)	350 (250–490)
11 N^6-2-Phenethyl-	16.1 (12.3–21.0)	3.5 (1.9–6.6)	28.7 (20.1–41.0)
12 N^6-2-(3-Pyridylethyl)-	10.4 (7.3–14.9)	4.8 (3.6–6.4)	76 (47–123)
13 N^6-2-(4-Pyridylethyl)-	16.2 (8.9–29.4)	2.7 (1.0–7.0)	82 (64–106)
14 N^6-2-(2-Thienylethyl)-	6.8 (2.5–17.5)	1.9 (0.8–5.0)	13.3 (9.2–19.4)
15 N^6-$CH_3NHCOCH_2C_6H_4$-	13.2 (7.9–21.8)	3.2 (2.2–4.6)	32.9 (28.5–38.1)
16 N^6-$CH_3C_6H_4NHCOCH_2C_6H_4$-	1.7 (0.77–3.9)	1.0 (0.93–1.1)	7.7 (6.2–9.5)
17 N^6-$H_2N(CH_2)_2NHCOCH_2C_6H_4$-NHCOCH$_2C_6H_4$- (ADAC)	1.3 (1.0–1.7)	0.39 (0.27–0.57)	14.1 (13.6–14.7)
18 N^6-*R*-1-Phenyl-2-propyl- (R-PIA)	1.2 (0.89–1.5)	0.56 (0.45–0.70)	3.6 (3.1–4.1)
19 N^6-*S*-1-Phenyl-2-propyl- (S-PIA)	50 (44–57)	12.2 (9.1–16.3)	83 (59–117)
20 N^6-2-Methyl-2-propyl- (MePIA)	130 (110–160)	150 (130–180)	610 (410–890)
21 5′-*N*-Ethylcarboxamido- (NECA)	7.8 (5.4–11.2)	52 (40–68)	6.3 (5.5–7.3)
22 5′-*N*-Methylcarboxamido	64 (42–83)	770 (650–920)	70 (50–97)
23 5′-Carboxamido-	59 (46–75)	770 (520–1130)	60 (43–83)
24 2-Chloro-	7.5 (6.1–9.1)	92 (68–124)	11.7 (6.1–22.4)
25 2-Phenylamino-	400 (220–730)	1400 (950–2100)	540 (510–580)
26 N^6-Cyclohexyl-NECA	0.57 (0.35–0.93)	1.4 (1.1–1.9)	1.1 (0.80–1.5)
27 N^6-*R*-1-Phenyl-2-propyl-NECA	0.51 (0.42–0.64)	1.1 (0.69–1.7)	4.6 (3.2–6.5)
28 N^6-*S*-1-Phenyl-2-propyl-NECA	9.8 (6.7–11.5)	8.9 (6.9–11.6)	41 (37–47)

Binding of 1 nM [^{3}H]PIA was measured at 37°C. Values are geometric means with 95% confidence limits from n = 4–6 with rat and n = 3 with calf and guinea pig cerebral cortex membranes, respectively

A1 receptors of rat and calf brain (table 1). The only exception is N^6-1-methylcyclopentyladenosine (**6**), which is nearly 5-fold more potent at A1 receptors of rat brain than at calf brain receptors. The affinities of these compounds for A1 receptors in guinea pig cerebral cortex membranes are at most only slightly lower than those at A1 receptors in rat and calf brain.

In the series of N^6-aryl (**9**) and N^6-aralkyl (**10**, **11**) analogs, however, marked species differences in affinities for A1 receptors are evident. The presence of a phenyl moiety in the N^6 substituent of adenosine analogs results in a 3–5-fold higher affinity at the calf brain A1 receptor than at the rat brain receptor: this increase in affinity is independent of the distance separating the aromatic ring from the N^6-nitrogen (**9–11**). The affinities of these aryl and aralkyl analogs for A1 receptors of guinea pig brain are even lower. The three heteroaralkyl analogs (**12–14**) are also more potent at the calf brain A1 receptor than at the rat brain receptor. The N^6-2-(4-pyridylethyl)adenosine (**13**) is notable in being 6-fold more potent at calf than rat brain. The affinities of these heteroaralkyl analogs are even lower for A1 receptors of guinea pig brain, at which receptors **13** is 30-fold less potent than at calf brain receptors.

A functionalized congener approach has recently been applied to adenosine to yield compounds such as **15–17** [7]. These functionalized congeners are also N^6-aryladenosines. The methylamido derivative of N^6-(4-carboxymethyl)phenyladenosine (**15**) is about 4-fold more potent in calf brain than in rat brain. The incorporation of an additional aryl ring (**16**) results in a very potent analog, which has, however, the same affinity in rat and calf brain. Further extension of the functionalized chain results in a compound with a terminal $-NHCH_2CH_2NH_2$ group (ADAC, **17**), which is about 3-fold more potent in calf than in rat brain. These functionalized congeners are less potent in guinea pig brain than in rat brain.

Stereoselectivity for N^6-aralkyladenosine with a chiral carbon attached to the N_6 nitrogen is noted at both A1 and A2 adenosine receptors [1,2]. Indeed, the higher stereoselectivity of *R*- and *S*-PIA (**18**,**19**) at A1 receptors has been widely utilized in attempts to define adenosine receptor subclasses. The *R/S* ratio for PIA is about 40-fold in rat brain, but only 20-fold in calf brain, mainly because of a 4-fold increase in affinity for *S*-PIA (**19**) in calf brain compared to an only 2-fold increase for *R*-PIA (**18**). The *R/S* activity ratio for PIA is 23 in guinea pig brain, with both isomers being less active in guinea pig than in rat brain. The presence of another methyl group on the carbon attached to the N^6 nitrogen in PIAs removes chirality and creates a tertiary carbon attached to the N^6 nitrogen in MePIA (**20**). MePIA has the same affinity in rat and calf brain, thereby being 2.5- and 12-fold, respectively, less potent than *S*-PIA (**19**). It is much less potent in guinea pig brain. All of the N^6-aryl and N^6-aralkyl analogs (**9–20**) have lower affinity in guinea pig than in rat brain. All but one (MePIA, **20**) have lower affinity for rat brain than for calf brain. The most striking examples of species differences are for N^6-phenyladenosine (**9**), the heteroaralkyl analog **13** and for the functionalized congener (ADAC, **17**), which are respectively 30- to 36-fold less potent in guinea pig brain than in calf brain.

NECA (**21**) was the most potent carboxamide derivative tested. NECA (**21**) is about 8–14-fold more potent than the 5′-*N*-methylamido analog (**22**) and the unsubstituted 5′-amido analog (**23**) in rat, calf and guinea pig brain. The carboxamides **22** and **23** are equivalent to each other in activity in the three species. However, the carboxamide derivatives (**21–23**) are 7–13-fold less potent in calf brain than in rat and guinea pig brain. This is of particular interest, because the different order of potency for *R*-PIA and NECA has been widely used for the definition of the subclass of adenosine receptor involved in a particular function [1,2]. *R*-PIA (**18**) is 6.5-fold more potent than NECA (**21**) at A1 receptors of rat brain, 93-fold in calf brain and only 1.8-fold more potent in guinea pig brain.

Alterations in the C2 position of the purine ring lead to potent adenosine analogs. 2-Chloroadenosine (**24**) is as potent as NECA (**21**) at A1 receptors of rat brain. With a K_i of 92 nM, 2-chloroadenosine is about 12-fold less potent in calf brain than in rat brain. 2-Phenylaminoadenosine (**25**) is of particular interest because it is the first adenosine analog found to be significantly more potent at A2 receptors than at A1 receptors [13]. This analog (**25**) is equally potent at A1-binding sites of rat and guinea pig brain, but about 3-fold less potent in calf brain. Therefore, at least some adenosine analogs with substitutions at

C5 of the ribose ring or at C2 of the purine ring have reduced affinity to A1-binding sites in calf cerebral cortex.

Recently, a new group of N^6-substituted NECA derivatives has been introduced for receptor classification [11,14]. At the A2 receptor of dog coronary artery the potency of the N^6-modified NECA analogs is similar to that of the N^6-substituted adenosines. Thus, an N^6 substituent exerts the same effect on an adenosine-5′-uronamide as on an adenosine, but completely suppresses the potential of the amide function to contribute to vasoactivity [11]. In contrast, at the adenylate cyclase-coupled A2 receptors of rat pheochromocytoma (PC12) cells and human platelets, the 5′-ethylcarboxamido group can modestly increase the potency of certain N^6-substituted adenosines, but in no case is the N^6-substituted NECA more potent than NECA [11,14]. At the A1-binding site of rat brain, the 5′-ethylcarboxamido group (compounds **26–28**) increases potency about 2–5-fold over that of the N^6-substituted adenosine. In calf brain the N^6-substituted NECA derivatives have similar affinity to the N^6-substituted adenosines, whereas in guinea pig brain an increase in affinity of about 2-fold occurs for the N^6-cyclohexyl- (**26**) and the N^6-*S*-1-phenyl-2-propyl- (**28**) NECA derivatives. Therefore, the N^6-substituted NECA derivatives appear potentially very useful for receptor classification, because the presence of the N^6 substituent enhances or does not change activity at A1 receptors, while reducing activity of NECA at the A2 receptors [11,14].

Theophylline (1,3-dimethylxanthine, **29**), a well known antagonist at adenosine receptors, has similar affinity in rat and calf brain (table 2). It is somewhat less potent in guinea pig brain. Incor-

Table 2

Inhibition of [^{3}H]PIA binding to cerebral cortex membranes by xanthine derivatives

Xanthine	Inhibition [^{3}H]PIA binding (K_i, nM)					
	Rat		Calf		Guinea pig	
29 Theophylline	12800	(10800–15300)	13500	(7000–26000)	24700	(19800–30800)
30 8-Phenyltheophylline	76	(58–98)	7.6	(4.0–14.4)	1540	(910–2610)
31 8-*p*-Sulfophenyltheophylline	1000	(770–1360)	300	(190–270)	10100	(7250–13950)
32 8-Cyclopentyltheophylline	510	(400–660)	54	(42–69)	1200	(980–1500)
33 8-Phenylaminotheophylline	5300	(3200–8900)	1200	(500–2900)	21700	(12800–36600)
34 Caffeine	44000	(31000–63000)	44000	(27000–73000)	47000	(36000–62000)
35 8-Phenylcaffeine	14700	(12200–17700)	8800	(6300–12600)	25200	(17800–35600)
36 1,3-Dipropylxanthine	710	(670–750)	340	(230–510)	1310	(1000–1720)
37 1,3-Dipropyl-8-phenylxanthine	10.0	(5.5–18.2)	0.22	(0.07–0.69)	20.9	(16.3–26.9)
38 8-(*p*-Sulfophenyl)-1,3-dipropylxanthine	140	(110–200)	32	(20–52)	810	(390–1650)
39 1,3-Dipropyl-8-(*p*-$H_2N(CH_2)_2$-$NHCOCH_2$-OC_6H_4)-xanthine (XAC)	1.3	(1.0–1.7)	0.15	(0.09–0.24)	16.5	(9.6–28)
40 1,3-Dipropyl-8-cyclopentylxanthine	1.2	(0.71–1.9)	0.29	(0.21–0.39)	3.9	(2.6–5.9)
41 1,3-Dipropyl-8-cyclohexylxanthine	1.4	(0.91–2.0)	0.21	(0.11–0.43)	4.7	(3.4–6.6)
42 1-Propargyl-3,7-dimethylxanthine	11000	(9000–13000)	16400	(15100–17800)	25800	(22400–29700)
43 1,3-Dimethyl-7-propylxanthine	19800	(15200–25700)	18200	(16200–20500)	33300	(19000–58000)
44 1,3-Dipropyl-7-methylxanthine	3400	(3500–4400)	2400	(1200–4700)	6200	(4700–8100)
45 3-Isobutyl-1-methylxanthine	6200	(5000–7800)	4400	(3700–5200)	8600	(6200–11800)

Binding of 1 nM [^{3}H]PIA was measured at 37°C. Values are geometric means with 95% confidence limits from n = 3 experiments

poration of an 8-phenyl group into theophylline (**30**) enhances affinity about 170-fold in rat brain and about 1800-fold in calf brain, as reported in [10]. In guinea pig brain, however, the 8-phenyl group increases affinity by only about 9-fold. 8-Phenyltheophylline (**30**) is thus 11-fold more potent in calf than rat brain and 200-fold more potent in calf than guinea pig brain. 8-*p*-Sulfophenyltheophylline (**31**) is about 3-fold more potent in calf than rat brain and about 30-fold more potent in calf than guinea pig brain. The higher affinity of an 8-substituted xanthine for A1 receptors in calf brain compared to rat or guinea pig brain is also observed with 8-cyclopentyltheophylline (**32**). This xanthine is only 2-fold less potent in guinea pig brain than in rat brain. Separation of the phenyl group of 8-phenyltheophylline by an amine nitrogen so as to yield 8-phenylaminotheophylline (**33**) yields a weak antagonist that shows a 4.5-fold difference in affinity between calf and rat brain, and a 4-fold difference between rat and guinea pig brain.

Caffeine (**34**) shows the same affinity for A1 receptor binding sites of rat, calf and guinea pig brain, respectively (table 2). In this case, incorporation of an 8-phenyl group increased affinity by 5-fold in calf, 3-fold in rat and 2-fold in guinea pig brain, respectively. Thus, effects of an 8-phenyl group are less species dependent for caffeine than for theophylline. Replacement of the methyl groups of theophylline with propyl groups to yield 1,3-dipropylxanthine (**36**) results in an increase in affinity of almost 20-fold in rat and guinea pig brain and a 40-fold increase in calf brain. This xanthine, like theophylline and caffeine, thus shows only slight species differences for interactions with brain A1 receptors. Remarkable further increases in activity occur with 1,3-dipropylxanthines having 8-aryl substituents. Compound **37** is 60–70-fold more potent than 1,3-dipropylxanthine (**36**) in rat and guinea pig brain and more than 1500-fold more potent in calf brain. As recently shown, combinations of 1,3-dipropyl and 8-phenyl substituents can lead to selectivity for A1 receptors compared to A2 receptors [12,15,16].

Since many of the potent and/or selective 8-phenylxanthines have very low water solubility, which limits their usefulness in vivo because of low drug availability, polar substituents such as *p*-sulfo groups have been introduced into the 8-phenyl ring to increase water solubility [13]. As can be seen from compounds **31** and **38**, these substituents also markedly reduce the potency at brain A1 receptors from all three species. As with other 8-substituted xanthines, the same profile of potency pertains for the 8-*p*-sulfophenylxanthines, namely calf > rat > guinea pig.

XAC (**39**) is a functionalized congener derived from 1,3-dipropyl-8-(4-hydroxyphenyl)xanthine [17]. It exhibits very high affinity for A1 receptors of rat and calf brain. As in the case of the functionalized congener ADAC (**17**) in the agonist series, the high affinity of XAC appears due to the presence of the distal ammonium moiety. The enhancing effect of a positively charged moiety on A1 receptor affinity is also noted in conjugates with amino acid moieties [18]. Tritiated XAC represents the first truly satisfactory antagonist radioligand for adenosine receptors [6,17]. It is most potent in calf brain, being 9-fold more potent in this species than in guinea pig.

1,3-Dipropyl-8-cyclopentylxanthine (**40**) and 1,3-dipropyl-8-cyclohexylxanthine (**41**) are antagonists with very high affinity for A1 adenosine receptors. As with 1,3-dipropyl-8-phenylxanthine (**37**), compounds **40** and **41** have a 3–5-fold higher affinity to calf brain A1 receptors compared to those of rat brain and a 3–4-fold lower affinity in guinea pig brain adenosine receptors compared to rat brain.

1-Propargyl-3,7-dimethylxanthine (**42**) has recently been described as a xanthine derivative with some selectivity for A2 adenosine receptors [13,17]. The presence of the polar propargyl group contributes to a 2–3-fold increase in potency compared to caffeine in calf and guinea pig brain, while having no effect in rat brain. 1,3-Dimethyl-7-propylxanthine (**43**) is another A2-selective antagonist [13,16]. It is about 2-fold more potent than caffeine at A1 receptors of calf and rat brain, while being only slightly more potent than caffeine at A1 receptors of guinea pig brain. 1,3-Dipropyl-7-methylxanthine (**44**) and 3-isobutyl-1-methylxanthine (**45**) are relatively potent in all three species. These xanthines along with theophylline (**29**), caffeine (**34**), 1,3-dipropylxanthine (**36**) and the other two xanthines (**42,43**) that lack an 8-substituent have nearly equivalent activity at A1 receptors of rat, calf and guinea pig brain.

4. DISCUSSION

The present results provide strong evidence for well-defined species differences in the recognition site on brain A1 receptors for both agonists and antagonists. The differences are strongly dependent on specific molecular features as follows:

4.1. *Agonists*

(i) The presence of a phenyl or heteroaryl moiety in the N^6 substituent of adenosine analogs greatly enhances affinity at the calf brain A1 receptor resulting in agonists that can be as much as 30-fold more potent in calf than in guinea pig brain. Potency is intermediate in rat brain. Thus, a change or alteration in the structure of the A1 receptor apparently has occurred in calf brain that enhances interactions with an aryl or heteroaryl ring. (ii) The presence of a 5′-ethylcarboxamido moiety results in a great reduction in potency at A1 receptors of calf brain compared to rat and guinea pig brain. This lowering of affinity in calf brain by the 5′-carboxamido moiety is overcome by the presence of an N^6 substituent. (iii) The presence of a 2-substituent results in a slight (compound **25**) to large (compound **18**) reduction in potency at the A1 receptor of calf brain compared to rat and guinea pig brain.

4.2. *Antagonists*

(i) The presence of an 8-substituent (either aryl or cycloalkyl) greatly enhances affinity at the calf brain A1 receptor resulting in antagonists that can be as much as 200-fold more potent in calf than in guinea pig brain. Potency is intermediate in rat brain. Xanthines, such as theophylline, caffeine, 1,3-dipropylxanthine, compounds **42–44** and IBMX are equipotent or nearly so in the three species. It is tempting to speculate that the alteration in the A1 receptor that affects potency for N^6-aryl, N^6-aralkyl, or N^6-heteroaralkyl substituted adenosines is the same alteration that affects potency of 8-aryl- or 8-cycloalkylxanthines: i.e. binding of the N^6 substituent of an adenosine occurs in a similar manner or at the same loci as binding of the 8-substituent of a xanthine.

Regardless of the nature of the alterations in the brain A1 receptor from different species, the results provide a caution against extrapolating profiles of potencies for agonist and antagonist from one species to another. Whether the species differences in brain A1 receptors also occur with A1 receptors of fat or heart cells, and whether recognition sites for A2 receptors are conserved in different species remain to be investigated.

ACKNOWLEDGEMENT

D.U. is on leave from the Pharmakologisches Institut der Universität Heidelberg with support of the Deutsche Forschungsgemeinschaft (Uk 4/1-1).

REFERENCES

[1] Daly, J.W. (1982) J. Med. Chem. 25, 197–207.
[2] Daly, J.W. (1985) Adv. Cyclic Nucleotide Protein Phosphorylation Res. 19, 29–46.
[3] Collis, M.G. (1984) in: Purines: Pharmacology and Physiological Roles (Stone, T.W. ed.) pp.75–84, MacMillan, London.
[4] Bruns, R.F., Daly, J.W. and Snyder, S.H. (1980) Proc. Natl. Acad. Sci. USA 777, 5547–5551.
[5] Murphy, K.M.M. and Snyder, S.H. (1982) Mol. Pharmacol. 22, 250–257.
[6] Jacobson, K.A., Ukena, D., Kirk, K.L. and Daly, J.W. (1986) Proc. Natl. Acad. Sci. USA 83, 4089–4093.
[7] Jacobson, K.A., Kirk, K.L., Padgett, W.L. and Daly, J.W. (1985) J. Med. Chem. 28, 1341–1346.
[8] Ukena, D., Jacobson, K.A., Kirk, K.L. and Daly, J.W. (1986) FEBS Lett. 199, 269–274.
[9] Kusachi, S., Thompson, R.D., Bugni, W.J., Yamada, N. and Olsson, R.A. (1985) J. Med. Chem. 28, 1636–1643.
[10] Kusachi, S., Thompson, R.D., Bugni, W.J., Yamada, N., Daly, D.T. and Olsson, R.A. (1986) J. Med. Chem. 29, 989–996.
[11] Olsson, R.A., Kusachi, S., Thompson, R.D., Ukena, D., Padgett, W. and Daly, J.W. (1986) J. Med. Chem. 29, 1683–1689.
[12] Daly, J.W., Padgett, W., Shamim, M.T., Butts-Lamb, P. and Waters, J. (1985) J. Med. Chem. 28, 487–492.
[13] Daly, J.W., Padgett, W.L. and Shamim, M.T. (1986) J. Med. Chem. 29, 1305–1308.
[14] Ukena, D., Olsson, R.A. and Daly, J.W. (1986) Can. J. Physiol. Pharmacol., submitted.
[15] Bruns, R.F., Daly, J.W. and Snyder, S.H. (1983) Proc. Natl. Acad. Sci. USA 80, 207–208.

[16] Daly, J.W., Padgett, W.L. and Shamim, M.T. (1986) J. Med. Chem. 29, 1520–1523.
[17] Ukena, D., Daly, J.W., Kirk, K.L. and Jacobson, K.A. (1986) Life Sci. 38, 797–807.
[18] Jacobson, K.A., Kirk, K.L., Padgett, W.L. and Daly, J.W. (1986) Mol. Pharmacol. 29, 126–133.
[19] Ukena, D., Jacobson, K.A., Kirk, K.L. and Daly, J.W. (1986) FEBS Lett. 199, 269–274.
[20] Ukena, D., Shamim, M.T., Padgett, W.L. and Daly, J.W. (1986) Life Sci. 39, 243–250.