Biochemical Pharmacology, Vol. 30, No. 21, pp. 2971–2977, 1981.
Printed in Great Britain.

0006-2952/81/212971-07 $02.00/0

VASOACTIVITIES OF ADENOSINE ANALOGUES IN TROUT GILL (*SALMO GAIRDNERI* R.)

DIDIER A. COLIN and CLAUDE LERAY
Laboratoire de Physiologie comparée des Régulations, C.N.R.S., B.P. 20 CR, F-67037 Strasbourg-Cédex, France

(*Received* 22 *December* 1980; *accepted* 19 *May* 1981)

Abstract—Various adenosine analogues phosphorylated or not, modified at the purine ring level or in the carbohydrate moiety, have been tested for their ability to induce a vasoconstriction in the arterio-arterial vascular bed of the trout gill. The structural integrity of the purine ring and the *N*-glycosidic bond are required for activity. The *anti* conformation of the molecule is preferable to the *syn* conformation. The basicity and/or the hydrogen bonding capabilities at the 6-position are important for the potency of the molecule. Substitutions which decrease the basicity of the 1-position decrease the activity and substitutions which reinforce the basicity (2-Cl adenosine) increase it. Alterations of the furanose ring conformation and the reduction of the hydrogen bonding capabilities of the 2′- and 3′-hydroxyls decrease the potency of the compound. Some substitutions in the 5′-position intensify the haemodynamic response. Thus, 5′-ethyl carboxamide adenosine is one order of magnitude more potent than adenosine. The addition of more than one phosphate in 5′-position (ADP, ATP) favours the effectiveness of the compound but a further elongation of the phosphate chain does not improve its potency. The α-β configuration of the phosphate chain is essential for the physiological effect. IMP, adenylosuccinate and all cyclic necleotides are devoid of haemodynamic activity. These results sustain the hypothesis of the presence of specific vascular purinergic receptors in the trout gill.

It has been recently demonstrated that adenosine produces a vasoconstriction in the systemic vasculature of the trout gill [1, 2]. The site of action of adenosine (Ado) and phosphorylated derivatives has been located on the surface of the vascular cells of the trout gill [3].

The present experiments were performed to define the structure activity relationships of Ado in the vascular bed of the trout gill. Thus, the activities of various Ado derivatives were compared to the activity of Ado itself. A series of 78 Ado analogues substituted either on the purine ring or on the ribose moiety or both, was tested on the arterial vasculature of the trout gill. Preliminary reports of these studies have already been presented [4, 5].

MATERIALS AND METHODS

Rainbow trout (200–300 g) were acquired and maintained as previously described [2]. Isolated trout heads were prepared and perfused as previously described [3]. Temperature was 13 ± 0.5°.

The Ado analogues were injected in 50 μl solutions during 3.6 sec. Solutions were tested at 3×10^{-6}, 3×10^{-5}, 3×10^{-4} or 3×10^{-3} M according to the magnitude of the haemodynamic effect of each analogue. The responses of Ado and analogues were calculated as per cent of inhibition of the aortic outflow at the maximum of the effect [3] and then expressed as per cent of the response given by a solution of 3×10^{-5} M Ado injected on the same preparation. Each solution was tested 3 times on each of 4 different perfused trout heads and the results are the means of the values obtained on the 4 trout rounded into the whole numbers.

Adenosine analogues. Adenosine (Ado), 1-oxide Ado, 1-methyl Ado, tubercidin, 2-Cl Ado, all 6-deamino nucleosides, 6-methylamino purine riboside (PR), 6-furfurylamino PR, 2′-methoxy Ado, 2′-deoxy Ado, 3′-deoxy Ado, arabinofuranosyl adenine, 5′-sulphate-5′-deoxy Ado, AMP, Ado-2′-phosphate 5′-*O*-thio AMP, 5′-amido AMP, 2′-deoxy AMP, inosine-5′-phosphate (IMP), adenylosuccinate, 8-(6-aminohexyl)-amino AMP, diphospho-, triphospho- and cyclic nucleotides were purchased from Sigma Chemical Company (St. Louis, MO). Formycin B and 6-Cl PR-5′-phosphate were obtained from PL Biochemicals (Milwaukee), 6Δ2-isopentenylamino PR and 2′,3′-diacetyl from Aldrich-Europe Division (Belgium) and diAdo pentaphosphate from Boehringer Mannheim (France).

The following nucleosides and nucleotides were generous gifts: 1-ethyl Ado, 8-Br Ado, 2′,3′-isopropylidene Ado, 5′-Cl-5′-deoxy Ado, 5′-*S*-isobutyl Ado, 5′-deoxy Ado, *S* adenosyl homocysteine, *S* adenosyl methionine, 5′-tosyl Ado, 2-hydroxymethylene, 6-(9-adenyl) dioxane, 2-hydroxy,1-(9-adenyl)ethoxy, and 2-Cl,2′-hydroxy isopropane from Dr. M. Gero, C.N.R.S. (Gif-sur-Yvette, France); formycin from Dr. J. J. Fox, Sloan–Kettering Institute for Cancer Research (New York, NY); 2-azido Ado from Dr. N. J. Cusack, University of Cambridge (Cambridge, U.K.); 6-benzylamino PR and 6-benzylamino arabinofuranosyl adenine from Dr. J. L. Barascut, Laboratoire de Chimie bio-organique (Montpellier, France); 6-diphenylphosphoamino PR, 6-methyl, diphenylphosphoamino PR, and 5′-diphenyl AMP from Dr. W. Pfleiderer, University

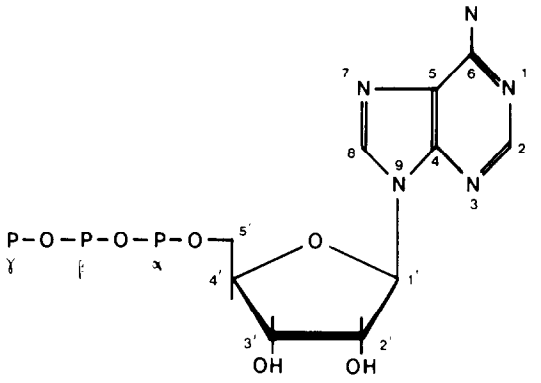

Fig. 1. Structure of ATP, illustrated in the *anti* conformation, showing the positions numbering utilized to characterise adenosine analogues.

of Konstanz (Konstanz, West Germany); 5′,8-cyclo Ado and 4′,5′-dehydro Ado from Dr. J. Zylber, C.N.R.S. (Thiais, France); 2′-Cl Ado, 3′-Cl Ado, 3′-amino Ado, 2′-azido Ado and 3′-azido Ado from Dr. H. Wiedner, Max-Planck Institute (Göttingen, West Germany); 5′-amino-5′-deoxy Ado and 5′-azido-5′-deoxy Ado from Dr. M. J. Robins, University of Alberta (Alberta, Canada); 5′-carboxylic acid methyl ester Ado, 5′-ethyl carboxamide Ado and 2′,3′-nitro, 5′-ethyl carboxamide Ado from Drs. Schick and Dittman, Byk Gulden Pharmazeutica (Konstanz, West Germany); 5′-carboxylic acid ethyl ester Ado from Dr. A. O. Geiszler (Abbot, IL).

2-(9 Adenyl)formyl methoxy, 3-hydroxy propionaldehyde was synthesized according to Khym *et al.* [6].

RESULTS

Adenosine (Ado) analogues modified in either the purine ring or the sugar moiety (Fig. 1) were examined for their haemodynamic activities on gill arterio-arterial circulation in the trout. Figure 2 shows an example of original registration curve. The transient effects of microinjections of Ado on the aortic outflow are identical to the effects previously described [3]. The responses to the analogues 2′-methoxy Ado and NAD taken as examples are compared to the Ado response. Although the amplitudes of the analogue responses are different and related to their concentrations, they exhibit the same type of agonist activity than Ado.

Modifications of the purine moiety (Table 1) at the 1-position (1-oxide Ado, 1-methyl Ado, 1-ethyl Ado) decrease the agonist activity. Tubercidin (Fig. 3) had a very slight agonist activity and formycin (Fig. 3) was an order of magnitude less active than Ado. Deamination of this latter analogue (formycin B) greatly reduced its agonist activity. The substitution of a Cl or an azido group at the 2-position of Ado either increased or did not affect the vasomotor action of the molecule.

Analogues in which a single H on the amino nitrogen was substituted retained agonist activity and were in some cases even more effective than Ado at the same concentration. The activity of these analogues was similar whether the substituent was aliphatic (methyl, ethyl, isopentenyl) or aromatic (furfuryl, benzyl, diphenyl-phospho). In contrast, 6-methyl, diphenylphosphoamino PR had an agonist action when it was tested at ten times the concentration of Ado. Replacement of the 6-amino group with a H, Cl, methoxy, thio, or methylthio group rendered the compound practically inactive since at a concentration of 3×10^{-3} M they were equipotent to Ado at 3×10^{-5} M. The replacement of the 6-amino group by a hydroxyl group (inosine or guanosine) rendered the compound practically inactive.

The cyclisation of the molecule (5′,8-cyclo-Ado) reduced the agonist activity of Ado and the substitution of a Br group at the 8-position produced a very large loss in the agonist activity.

Modifications of the ribose moiety (Table 2) involving the 2′- and 3′-positions were associated with substantial loss of activity. Both 2′- and 3′-deoxyadenosine were only slightly active at millimolar concentrations. Substitution at the 2′-position by a Cl, methoxy or azido group rendered the compound only active at 3×10^{-4} M. The action of Ado was more affected by the substitution at the 3′-pos-

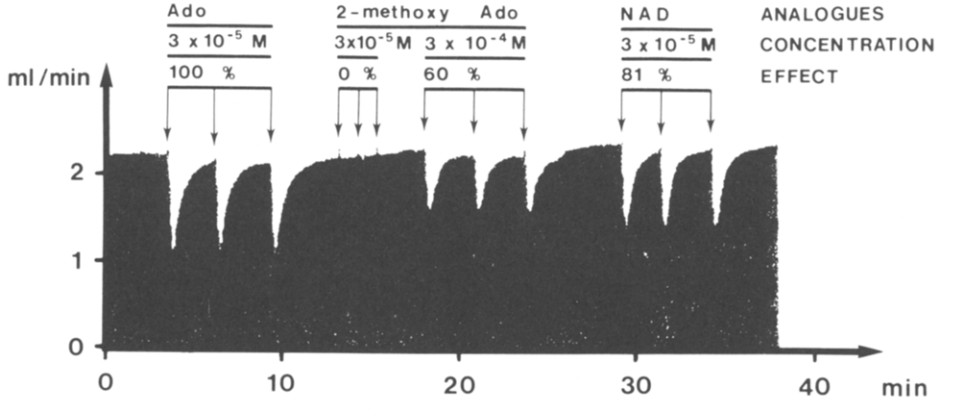

Fig. 2. Original registration curve of the aortic outflow of an isolated trout head preparation. The mean effect of 3 microinjections of 2′-methoxy Ado and NAD are compared to the mean effect of 3 microinjections of Ado taken as 100%. The mean effect of Ado, in this example, represents an inhibition of 48% of the aortic outflow.

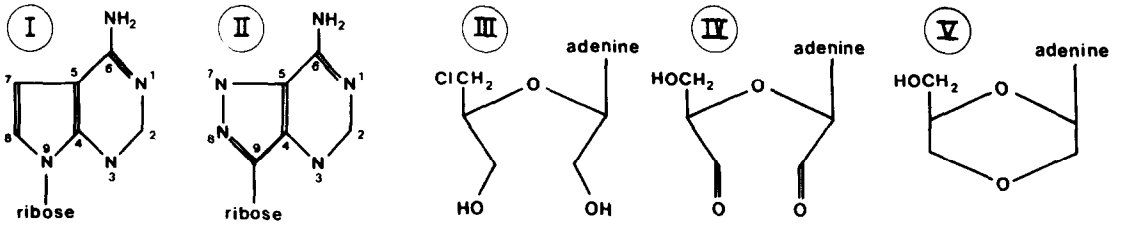

Fig. 3. Structure of some adenosine analogues modified either in the adenine ring (I:tubercidin; II:formycin) or in the ribose moiety III:2-(9-adenyl)formyl methoxy, 3-hydroxy propionaldehyde; IV:2-hydroxy,1-(9-adenyl)ethoxy,2-Cl,2'-hydroxy isopropane; V:2-hydroxy-methylene,6-(9-adenyl)dioxane.

ition by a Cl or an azido group than by an amino group. The compound 2',3'-diacetyl Ado was devoid of any activity, and 2',3'-isopropylidene Ado had a very small agonist activity, as did the two 2'-epimers tested, arabinofuranosyl adenine and its 6-benzyl derivative.

Among the 5'-deoxy derivatives tested, some were nearly equipotent to Ado (Cl, azido or *S*-isobutyl substitutions) while some others were only active at the millimolar level (sulphate, amino, homocysteine or methionine substitutions).

Substitution of Ado in 5'-position by an esterified COOH group led to an obvious decrease in potency. On the contrary, substitution by a carboxamide

Table 1. Haemodynamic effect of adenosine analogues substituted on the purine ring tested in microinjections on perfused trout gill (results are given in per cent of the adenosine response tested at 3×10^{-5} M)

	Test concentration		
	3×10^{-5} M	3×10^{-4} M	3×10^{-3} M
	Effect in percent		
Adenosine (Ado)	100		
N substitutions			
1-oxide Ado		122	
1-methyl Ado		121	
1-ethyl Ado		154	
tubercidin (7-deaza Ado)			33
formycin (8-aza,9-deaza Ado)		77	
formycin B			29
C substitutions			
2-Cl Ado	164		
2-azido Ado	106		
purine riboside (PR)			66
inosine			2
guanosine			8
6-methoxy PR			50
6-Cl PR			103
2-amino, 6-Cl PR			75
6-thio PR			15
6-methylthio PR			37
6-methylamino PR	114		
6-ethylamino PR	162		
6-Δ2-isopentenylamino PR	151		
6-furfurylamino PR	113		
6-benzylamino PR	157		
6-diphenylphosphoamino PR	90		
6-methyl, diphenylphosphoamino PR		102	
8-Br Ado			56
5',8-cyclo Ado		59	

Table 2. Haemodynamic effect of adenosine analogues substituted on the ribose moiety tested in microinjections on perfused trout gill (results are given in per cent of the adenosine response tested at 3×10^{-5} M)

	Test concentration			
	3×10^{-6} M	3×10^{-5} M	3×10^{-4} M	3×10^{-3} M
Analogues	Effect in percent			
Adenosine (Ado)		100		
2'-methoxy Ado			67	
2'-Cl Ado			97	
3'-Cl Ado				83
2'-deoxy Ado				58
3'-deoxy Ado				62
3'-amino Ado			37	
2'-azido Ado			62	
3'-azido Ado				54
2',3'-diacetyl Ado				0
2',3'-isopropylidene Ado				45
arabinofuranosyl Adenine				84
6-benzylamino arabinofuranosyl Adenine			14	
5'-Cl-5'-deoxy Ado		57		
5'-sulfate-5'-deoxy Ado				32
5'-amino-5'-deoxy Ado				66
5'-azido-5'-deoxy Ado		62		
5'-S-isobutyl-5'-deoxy Ado		67		
S-adenosyl homocysteine				27
S-adenosyl methionine				100
5'-carboxylic acid methyl ester Ado			79	
5'-carboxylic acid ethyl ester Ado			70	
5'-ethyl carboxamide Ado	110			
2',3'-nitro, 5'-ethyl carboxamide Ado				58
4',5'-dehydro Ado				113
5'-tosyl Ado				93
2-hydroxymethylene,6-(9-adenyl)dioxanne				53
2-hydroxy,1-(9-adenyl)ethoxy, 2-Cl, 2'-hydroxy isopropane				0
2-(9-adenyl)formyl methoxy, 3-hydroxy propionaldehyde				0

group greatly enhanced the agonist activity. Thus, 5'-ethyl carboxamide Ado was the most effective analogue tested on the isolated trout head preparation. With additional NO_2 substitutions in 2'- and 3'-positions a 100 fold increase in concentration was needed to detect an agonist activity.

The presence of a tosyl group in 5'-position resulted in a very slight activity (millimolar level). The modification of the ribose ring configuration (Fig. 3) rendered the compound either slightly active (4',5'-dehydro Ado, 2-hydroxymethylene,6-(9-adenyl) dioxane) or completely inactive (2-hydroxy, 1-(9-adenyl) ethoxy, 2-Cl, 2'-hydroxy isopropane and 2(9-adenyl) formyl methoxy, 3-hydroxy propionaldehyde).

The addition of a phosphate group (Table 3) at the 5'-position (AMP) did not affect the Ado activity but its addition at the 2'- or 3'-position decreased the agonist activity.

The tested substitutions at the 5'-position of Ado induced decreasing potencies according to the following order: AMP $\geq$ 5'-*O*-thio AMP > Ado 5'-monophosphoramidate > 5'-diphenyl AMP. The removal of the 2'-OH group resulted in a smaller decrease in potency for AMP than for Ado. Some substitutions on the purine ring of AMP either completely (IMP, 8-(6-aminohexyl)-amino 5'-AMP) or nearly completely (adenylosuccinate) destroyed the activity while the replacement of the 6-amino group by Cl induced a smaller inhibition. ADP and ATP were slightly more potent than Ado. The introduction of a methylene or an imido group between the β and γ phosphate groups of ATP decreased slightly its agonist activity whereas the introduction of a methylene group between the α and β phosphate groups of ADP or ATP resulted in a marked decrease in their activity. When a pyrophosphate group linked two Ado moieties (diAdo diphosphate), the activity was slightly enhanced while the prolongation of the phosphate chain (diAdo pentaphosphate) did not affect the activity. NAD and 5'-diphosphoribose Ado were practically equipotent to Ado, while NADP as Ado-2'-phosphate had the same effect at a ten-fold greater concentration.

Table 3. Haemodynamic effect of phosphorylated adenosine analogues tested in microinjections on perfused trout gill (results are given in per cent of the adenosine response tested at 3×10^{-5} M)

	Test concentration		
	3×10^{-5} M	3×10^{-4} M	3×10^{-3} M
Analogues	Effect in percent		
Adenosine (Ado)	100		
Monophosphates			
AMP	100		
Ado-2'-phosphate		103	
Ado-3'-phosphate		134	
5'-O thio AMP	76		
5'-amido AMP		62	
5'-diphenyl AMP			57
2'-deoxy AMP		52	
6-Cl PR-5'-phosphate		93	
inosine-5'-phosphate (IMP)			0
adenylosuccinate			12
8-(6-aminohexyl)-amino AMP			0
Diphosphates			
ADP	112		
α,β-methylene ADP			86
diAdo diphosphate	123		
5'-diphosphoribose Ado	95		
NAD	77		
Triphosphates			
ATP	122		
α,β-methylene ATP			55
β,γ-methylene ATP	95		
β,γ-imido ATP	96		
NADP		79	
Pentaphosphate			
diAdo pentaphosphate	93		
Cyclic nucleotides			
Ado-3', 5'-monophosphate (cAMP)			0
Ado-2', 3'-monophosphate			0
N6, 2'-O-dibutyryl cAMP			0

All the cyclic nucleotides tested (Ado-3',5'-monophosphate, Ado-2',3'-monophosphate, N6,2'-*O*-dibutyryl cAMP) were devoid of any activity.

DISCUSSION

Earlier studies [1] revealed that Ado and some of its natural derivatives had similar haemodynamic properties on trout gill. The present paper shows that besides natural compounds, various Ado analogues (nucleosides and nucleotides) also have vasoconstrictor effects on the arterial system of trout gill.

These results show that there is a wide range of vasomotor potencies among the 78 compounds tested. While only one is much more active than Ado, 21 are more or less equipotent to Ado, 19 have the same effect but at a ten-fold concentration, 29 are more or less active at a hundred-fold concentration and 8 compounds are completely devoid of activity. With regard to the less active analogues, the presence of trace amounts of active contaminants in the injected solutions cannot be ruled out since the purity of the analogues was not checked before use.

It is likely that the 6-amino group is essential for haemodynamic activity. This requirement might be explained by the high basicity and/or the hydrogen bonding capabilities of this group [7–9]. When one of the two 6-amino hydrogens is substituted by a methyl, an ethyl, an isopentenyl, a furfuryl or a benzyl, the hydrogen bonding capabilities are decreased while the basicity is increased at this position. Simultaneously, the activity of the compound is reinforced. The 6-methylation of diphenylphosphoamino PR which suppresses all hydrogen bonding possibilities but not all the basicity of this position reduces the activity. On the other hand, the 6-carboxyl groups of adenylosuccinate render the compound completely inactive.

A certain degree of basicity of the 1-position seems to be required for interaction of adenosine with gill vascular smooth muscles. Some additions on the 1-position (oxygen, methyl, ethyl) reduce its basicity and, concurrently, the activity. According to the

electrophilic substitution rules [10], the addition of Cl on the 2-position increases the basicity of the 1-position and potentiates the compound. The analogue 2-Cl Ado is also a more potent agonist than Ado on mammalian smooth muscles [11, 12]. The presence of an azide on the 2-position has no particular consequence on the activity.

The requirement of the *N*-glycosidic bond and the structural integrity of the purine ring is demonstrated by the low activities of formycin and 7-deaza Ado, respectively.

The capacity of Ado to interact with the gill vascular system of the trout may depend not only on the presence and the configuration of its different functional groups but on its conformation as well. Thus, although 5′-8-cyclo Ado which has a high *anti* conformation was not a very potent agonist, the very low biological activity of 8-Br Ado which has a *syn* conformation seems to demonstrate that the *anti* conformation is preferable for agonist activity. This stereochemical interpretation has already been proposed by Hampton, Harper and Sasaki [12] for Ado utilizing enzymes and by Olsson, Gentry and Snow [13] for Ado transport requirements.

This study shows that any alteration of the sugar moiety involving either the furanose ring conformation or the presence of the 2′- and 3′-hydroxyls is associated with a decrease or a complete loss of vasomotor activity. The erythro configuration of the 2′- and 3′-hydroxyls and concurrently their hydrogen bonding capabilities are also of great importance for the vasomotor activity of Ado. Considering the results obtained with different 2′- and 3′-substitutions, the 3′-hydroxyl appears as important for agonist activity as the 2′-hydroxyl, although some substitutions are more tolerable in the 2′-position.

Although 5′-deoxy Ado was not available, the results obtained with different 5′-substituted nucleotides suggest that the 5′-hydroxyl is relatively less important than the secondary hydroxyls for agonist activity. The presence of carboxyl groups in 5′-position considerably alters the potentiality of the compound. This alteration is less severe when the carboxyl group is esterified, but an additional substitution in 2′- and 3′-positions which increases the activity of the compound in dog coronaries [14] decreases its activity in the trout gill. Conversely, 5′-ethyl carboxamide Ado is the most effective of the analogues tested as it is in mammals [14].

If the addition of a phosphate group in the 5′-position does not alter the agonist activity of the Ado molecule, the addition of a phosphate group in 2′- or 3′-positions induces a decrease of activity as any other addition in these positions. The addition of a phosphate group in the 2′-position of NAD or Ado results in the same decrease of agonist activity. However, the weak activities of some Ado analogues (2′-deoxy Ado, 6-Cl PR) are reinforced by a further phosphorylation in 5′-position.

Among natural nucleotides, AMP, ADP, ATP and NAD are at least as potent as Ado but IMP and adenylosuccinate are inactive even at the millimolar concentration.

The integrity of the hydroxyl groups of the 5′-phosphate seems to be required for activity since the substitution of both hydroxyls (5′-diphenyl AMP) produces a greater reduction than when only one hydroxyl is substituted (5′-amido AMP).

The potencies of natural adenylic compounds observed in this study are in the order previously described [3]. These results suggest that the elongation to three phosphates of the phosphate chain favours the vasomotor response. A further prolongation of the chain (diAdo pentaphosphate) does not enhance the vasomotor activity as it can be expected according to the results of Raberger, Schütz and Kraupp [14] on diAdo derivatives with polymethylene chains. The configuration of the phosphate chain seems important, mainly in the α- and β-locations. It seems likely that the lengthening of the distance between the α and β phosphorus by introduction of a $-CH_2-$bridge (α,β-methylene ADP, α,β-methylene ATP) accounts for the observed decrease of activity. The same lengthening (β,γ-methylene ATP) in the β- and γ-locations does not alter significantly the agonist activity. This effect was already observed on synaptic transmission in the cerebral cortex [15]. Furthermore the γ-phosphate can be replaced by Ado, a ribose or a nicotinamide ribose group since diAdo diphosphate, 5′-diphosphoribose Ado and NAD are approximately equipotent to ADP.

As the 3 cyclic nucleotides tested are completely inactive, it can be assumed that the 3′, 5′- or the 2′, 3′-cyclisation prevents the access of the compound to the site of action and that no active metabolites are released by hydrolysing enzymes. As a matter of fact, neither tissue uptake nor degradation of ^{3}H cAMP could be detected in the trout perfused head preparation (unpublished results). The dibutyryl derivative of cAMP is also inactive in the isolated perfused guinea pig heart [16].

The higher potency of some Ado analogues, compared to that of Ado, can be interpreted as an increase of affinity for the site of action, a reduction of uptake and/or enzymic catabolism [17]. Taking into account the coronary dilator potencies of a series of Ado analogues, Angus *et al.* [11] suggested that enzymic inhibition was probably not the sole mode of action, although being a contributing factor. It seems likely that the strong activity of 5′-ethyl carboxamide Ado is mainly due to an increase of affinity. On the other hand, a part of the activity of 2-Cl Ado might be assigned to an inhibition of Ado deaminase [18]. The potency of some analogues (AMP, ADP, ATP, NAD, 5′-diphosphoribose) may result from either an intrinsic action of these molecules or a rapid enzymic hydrolysis into Ado. Earlier studies about haemodynamic effect of AMP on gill trout have shown that dephosphorylation was not a necessary prerequisite to agonist activity [3].

The weak agonist potency of some tested analogues and the inactivity of some others might result from large alterations of the basic features of the compound such that the molecule no longer fits the site of action and/or an increase of steric hindrance.

The present work shows that a number of Ado analogues with structural modifications have a constrictor activity on the gill vascular bed of trout. The necessary molecular features for Ado activity revealed by this study: integrity of purine and furanose rings, basicity of 1- and 6-positions, hydrogen

bonding at 6-, 2- and 3-positions, *N*-glycosidic bond and *anti* conformation are generally involved in other systems where steric requirements for Ado activity have been studied: coronary dilation [11, 17], intestinal smooth muscle relaxation [8, 9], ADP induced platelet aggregation [19], Ado membrane carrier in the dog heart [13], cAMP accumulation in the myocardium [7], in the brain or in human fibroblasts [21] and depression of synaptic transmission in the cerebral cortex [15].

The hypothesis of specific 'purinergic receptors' in the gill vascular system of rainbow trout formulated on the grounds of physiological experiments [3] is strongly supported by the findings of the present study. Whether the origin of the mediator is vascular cells themselves or purinergic nerves [22] is not yet elucidated.

Acknowledgements—The authors wish to thank Dr. M. S. Haswell for correcting the manuscript and Mr. A. Hubsch for his excellent technical assistance.

REFERENCES

1. D. A. Colin and C. Leray, *C. R. Acad. Sci., Paris,* **284**, 1191 (1977).
2. D. A. Colin, R. Kirsch and C. Leray, *J. comp. Physiol.* **130**, 325 (1979).
3. D. A. Colin and C. Leray, *Pflüg. Arch.* **383**, 35 (1979).
4. D. A. Colin and C. Leray, *IIIème Table Ronde internationale. Nucléosides, Nucléotides et leurs applications biologiques.* Montpellier, France (1978).
5. D. A. Colin and C. Leray, *J. Physiol., Paris,* **75**, 43A (1979).
6. J. X. Khym and W. E. Cohn, *J. Am. chem. Soc.* **82**, 6380 (1960).
7. M. Huang and G. I. Drummond, *Biochem. Pharmac.* **25**, 2713 (1976).
8. S. W. Leslie, J. L. Borowitz and T. S. Miya, *J. pharm. Sci.* **62**, 1449 (1973).
9. S. McKenzie, R. Frew and H. P. Baer, *Eur. J. Pharmac.* **41**, 183 (1977).
10. J. March, *Advanced Organic Chemistry,* 2nd edition. McGraw-Hill, London (1968).
11. J. A. Angus, L. B. Cobbin, R. Einstein and M. H. Maguire, *Br. J. Pharmac.* **41**, 592 (1971).
12. A. Hampton, P. J. Harper and T. Sasaki, *Biochemistry* **11**, 4736 (1972).
13. R. A. Olsson, M. K. Gentry and J. A. Snow, *Biochim. biophys. Acta,* **311**, 242 (1973).
14. G. Raberger, W. Schütz and O. Kraupp, *Archs int. Pharmacodyn. Thér.* **230**, 140 (1977).
15. J. W. Phillis, J. P. Edstrom, G. K. Kostopoulos and J. R. Kirkpatrick, *Can. J. Physiol. Pharmac.* **57**, 1289 (1979).
16. R. Bünger, F. J. Haddy and E. Gerlach, *Pflüg. Arch.* **358**, 213 (1975).
17. L. B. Cobbin, R. Einstein and M. H. Maguire, *Br. J. Pharmac.* **50**, 25 (1974).
18. C. Leray, J. P. Raffin and C. Winninger, *Comp. Biochem. Physiol.* **62**, 31 (1979).
19. K. C. Agarwal and R. E. Parks, *Biochem. Pharmac.* **28**, 501 (1979).
20. M. Huang and J. W. Daly, *J. med. Chem.* **15**, 458 (1972).
21. R. F. Bruns, *Can. J. Physiol. Pharmac.* **58**, 673 (1980).
22. G. Burnstock, *Pharmac. Rev.* **24**, 509 (1972).