resin was transferred to a column, washed with water, and developed with 1.3 liters of 5% ammonium hydroxide. The eluate was concentrated *in vacuo* to 25 ml, treated with active charcoal (5 g), and added dropwise to 1 liter of ethanol stirred at 0°. The precipitation was repeated, yielding 7.18 g (57%) of III, mp 204–206° dec. Recrystallization from water–ethanol afforded an analytical sample, mp 214–215° dec. [lit. (1) mp 214°].

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Vasodilator Activity of Adenosine Analogs

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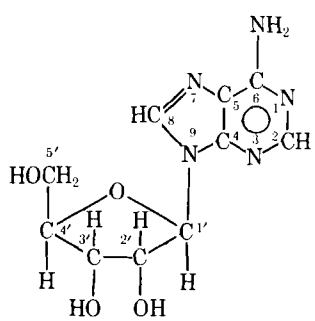
Abstract □ Various adenosine analogs were evaluated as smooth muscle vasodilators. The compounds were screened initially using a dog hindlimb preparation. Most analogs were less potent than adenosine. The three most potent compounds were tested for coronary vasodilator effects and duration of action on the isolated rabbit heart. 5′-Deoxy-5′-chloroadenosine and 5′-deoxy-5′-bromoadenosine were equipotent with adenosine but possessed a longer duration of action. 2′,3′,5′-Trideoxy-3′,5′-dichloroadenosine, an analog lacking both the 2′- and 3′-hydroxyl groups, had significant vasodilator activity.

Keyphrases □ Adenosine analogs, various—evaluated as smooth muscle vasodilators, dog hindlimb preparation □ Vasodilator activity—various adenosine analogs evaluated, dog hindlimb preparation □ Structure–activity relationships—various adenosine analogs evaluated as smooth muscle vasodilators, dog hindlimb preparation

The coronary vasodilator effects of adenosine (*Ia*) and its analogs have been recognized since 1929 (1). It has also been reported that these compounds cause the dilation of peripheral vessels (2, 3). It was suggested that adenosine is a physiological mediator of vasodilation (4). In all cases, the observed effects of adenosine are of short duration due to the rapid metabolism of the nucleoside to inosine (5) or phosphorylation to the corresponding nucleotide. Recently, Stein and coworkers (6, 7) reported that a series of esters and amides of adenosine-5′-carboxylic acid were potent cardiovascular agents that caused a marked increase in coronary sinus oxygen tension and prolonged

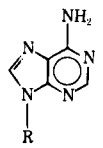


Ia

hypotension. These investigators postulated that these compounds act directly at an "adenosine receptor."

In an attempt to investigate the vasodilator activities of adenosine analogs further, a diverse series of adenosine analogs was synthesized and studied for peripheral activity in the hindlimb vasculature of the dog. Three of the most potent compounds were further studied on the Langendorff heart. The structural modifications included mono- and dihalo substituents in the sugar moiety, the extension of the 5′-position by one and two carbon atoms, an unsaturation in the carbon chain attached to the 5′-position, alterations in the sugar moiety, and a modification in the imidazole ring system. The vasodilatory effects of these compounds were compared to those of adenosine.

Table I—Potency Ratios of Adenosine Analogs on Perfused Hindlimb Preparation



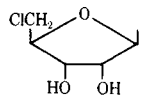
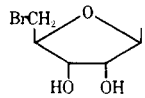
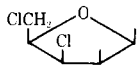
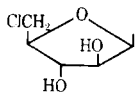
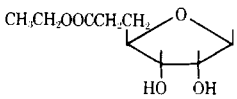
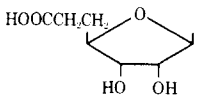
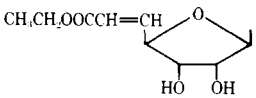
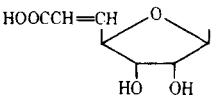
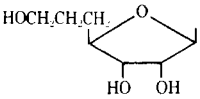
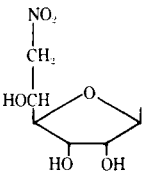
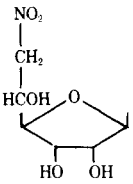
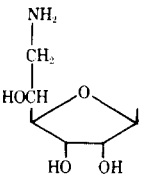
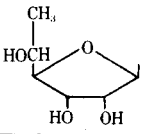
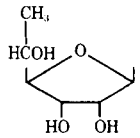
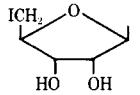
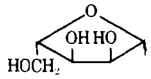
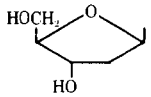
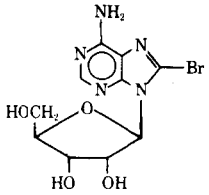
Compound	R	Potency[a]	Synthesis Literature Reference
Ib		0.810	8
II		0.360	8
III		0.160	9
IV		0.008	9
V		0.011	10
VI		0.009	10
VII		0.008	10
VIII		0.003	10
IX		0.012	10
X		0.006	10
XI		0.027	10
XII		0.025	10
XIII		0.038	10

Table I—*Continued*

Compound	R	Potency[a]	Synthesis Literature Reference
XIV		0.009	10
XV		0.049	—
XVI		0.011	11, 12
XVII		0.004	—
XVIII		0.0005	—

[a] Vasodilator potency relative to adenosine; n = 3 except for XVIII where n = 2.

EXPERIMENTAL

Chemistry—Compounds Ib, II–XIV, and XVI were prepared by literature methods (Table I). Compounds Ia[1], XVII[2], and XVIII[2] were obtained commercially. Compound XV was prepared by the acid hydrolysis of *N*-formyl-*O*-(2′,3′-isopropylidene)-5′-deoxy-5′-iodoadenosine[3].

Pharmacology—*Perfused Hindlimb Preparation*—All compounds were screened for vasodilator activity in the perfused hindlimbs of mongrel dogs. Briefly, the preparation utilizes the left hindquarter isolated from the circulation by cannulation of the distal iliac artery with polyethylene tubing. The limb is perfused at a constant flow rate, with blood withdrawn from a carotid artery and pumped with a constant flow pump. Adequacy of isolation is determined by turning off the pump and noting a residual pressure of 25–30 mm Hg and the lack of pulsatile flow. Under these conditions, a change in perfusion pressure is proportional to changes in vascular resistance.

Drugs were dissolved in 10 ml of 0.9% saline solution with 1.0 mg of tartaric acid for solubilization and injected directly into the perfusion tubing proximal to the cannula. All test compounds were compared to adenosine as the standard, and relative potencies were determined according to Finney (13). Since this procedure was utilized for screening, any compounds that were less than 0.100 as active as adenosine were not studied in the isolated Langendorff heart.

Langendorff Heart Preparation—New Zealand rabbits of either sex were sacrificed with a blow on the head, and the heart was rapidly removed and placed in a dish containing Feigen's solution (composition: 154.00 m*M* NaCl, 5.63 m*M* KCl, 0.82 m*M* $CaCl_2 \cdot 2H_2O$, 23.81 m*M* $NaHCO_3$, and 11.10 m*M* dextrose). This preparation was essentially the same as described by Long and Chiou (14), except that perfusion pressure and heart rate and force were continuously displayed on a recorder[4].

Parallel Line Assay—In this type of assay, several different compounds are compared to a standard compound for their ability to cause the test responses. The purpose of this assay is to define as precisely as possible the potency of the test compound in terms of the standard compound. This assay was particularly suitable since the major interest was in comparing the activities of the adenosine analogs with adenosine.

[1] Zellstoff-fabrik, Waldorf, Germany.
[2] Sigma.
[3] Gift of Dr. Saburo Fukui, Kyoto University, Kyoto, Japan.
[4] Beckman.

Table II—Relative Potencies of Adenosine and Analogs

Compound	n	Potency[a] (95% Confidence Interval)	Significance[b]
Adenosine	15	1.00	
I*b*	9	0.85[c] (0.36–1.88)	NS
	9	2.28[d] (0.99–8.37)	NS
II	10	1.59[c] (0.81–3.56)	NS
	10	2.32[d] (1.05–7.23)	*
III	8	0.18[c] (0.04–0.38)	*
	8	0.51[d] (0.06–1.69)	NS

[a] Potency relative to adenosine; 100.0 μg of adenosine caused coronary perfusion pressure to decrease 59.5 ± 3.8 mm Hg (mean ± *SEM*, n = 15). The high dose of adenosine (243 μg) had a duration of action of 0.76 ± 0.07 min. [b] Significance was determined from the 95% confidence interval. For details, see *Experimental*. NS = not significantly different from adenosine. * = significantly different from adenosine. [c] Relative vasodilator potency. [d] Relative duration of action.

This method is better than using the ratio of ED_{50}'s since at least two or three doses of each compound are used in the analysis.

For optimal results, dose–response curves are constructed for the test compounds and adenosine. From these curves, doses of the test drug that approximate the same response as adenosine are then used for the assay. In the interpretation of the analysis of variance and potency estimations, F values at the 0.05 level are always used. In calculating the fiducial limits, a tabulated t value at the 0.05 level was used. By definition, any potency estimation along with the 95% confidence limit (same as fiducial) is significantly different from the standard compound if the interval does not contain 1.00.

RESULTS AND DISCUSSION

Table I shows the potencies relative to adenosine in the hindlimb preparation. With the exception of 5′-deoxy-5′-chloroadenosine (I*b*), 5′-deoxy-5′-bromoadenosine (II), and 2′,3′,5′-trideoxy-3′,5′-dichloroadenosine (III), the compounds were much less potent than adenosine. Compound IV, the *ara*-analog of I*b*, was almost inactive. Compounds V and VII, the saturated and unsaturated homologs of the potent ethyl esters of adenosine-5′-carboxylic acids VI and VIII, respectively, were inactive.

Extension of the 5′-position of adenosine by two carbon atoms as in IX led to a considerable drop in activity. Introduction of a nitromethyl or aminomethyl moiety in the 5′-position of adenosine by two carbon atoms as in X–XII was detrimental to vasodilator activity. Similarly, the introduction of a methyl group in the 5′-position of adenosine as in XIII and XIV led to decreased activity. 5′-Iodoadenosine (XV) was virtually inactive compared to the 5′-chloro (I*b*) and 5′-bromo (II) analogs. Compound XVIII, which contains an 8-bromo substituent in the adenine moiety, was the least active compound. The low order of activity of XVIII could, in part, be attributed to its *syn*- rather than *anti*-conformation (15).

Table II shows the coronary vasodilator potencies and duration of action of I*b*, II, and III relative to adenosine in the Langendorff heart preparation. The activity and duration of action of I*b* and II confirm earlier reports (16, 17). This study and an earlier report (17) showed that both 2′-deoxyadenosine (XVII) and 3′-deoxyadenosine are relatively weak vasodilators. It is, therefore, interesting that III, which lacks both 2′- and 3′-hydroxyl groups and has a chloro substituent in the 3′-position, was significantly more potent than IV–XVIII.

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