BINDING OF ADENOSINE ANALOGS WITH TYPE A_1 PURINE RECEPTORS IN THE GUINEA PIG ILEUM

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For many years purine nucleosides have been regarded exclusively as precursors in the synthesis of high-energy phosphates, DNA, and RNA. However, it has been shown in recent years that purine compounds and, in particular, adenosine and its derivatives, monitor the physiological functions of the body through interaction with specific A_1- and A_2-adenosine receptors. Receptors of this type are widely represented in pre- and postsynaptic membranes of brain structures, in heart muscle, blood vessels, various parts of the small intestine, and so on [1, 3, 7].

One widely used method of studying affinity of agonists for receptors of a particular type is to use isolated animal organs. It has been shown, for instance, that inhibition of cholinergic transmission in the guinea pig ileum (GPI) is effected through type A_1 purine receptors, whereas contraction of the guinea pig vas deferens (GPVD) is effected through A_2-receptors [2, 6].

The aim of this investigation was to study the degree of affinity of stable adenosine derivatives (modified in position 6 of the purine ring) with purine receptors of GPI and GPVD.

EXPERIMENTAL METHOD

An isolated segment of GPI was placed in a bath containing Tyrode solution (volume 50 ml). The temperature of the solution was maintained between 34 and 34.5°C. Electrical stimulation was applied through a plate electrode (field stimulation) with the following parameters: pulse duration 1 msec, frequency 0.1 Hz, amplitude above threshold.

Mechanical contractions were recorded by a strain-gauge transducer under isometric conditions. The volume of the substances injected did not exceed 0.1 ml.

As a result of the experiments the concentration of the test substance which induced inhibition of contractions of GPI and GPVD by 50% of their initial amplitude (ED_{50}) was determined.

An isolated segment of GPVD 1.5 cm long was transferred into the same conditions as GPI, but electrical stimulation was not applied in this case. Contractions of GPVD were recorded isometrically during the action of agonists of adenosine receptors. A preparation of ATP was used as the standard.

EXPERIMENTAL RESULTS

The results showed that N^6-substituted adenosine derivatives have higher affinity for A_1 purine receptors than for the A_2 type [5]. The effect of known agonists of adenosine receptors, namely N^6-cyclohexyladenosine (CHA) and 6-DL-phenylisopropyl-adenosine (PIA), and also of adenosine derivatives synthesized in the Research Institute of Antibiotics and Medically Important Enzymes, on contractility of GPI and GPVD was studied. CHA in a dose of $1.7 \cdot 10^{-10}$ M and PIA in a dose of $5.4 \cdot 10^{-11}$ M were found to inhibit contractions of GPI by more than 50% in response to electrical stimulation. Preparation 2 had no effect on contractility of GPI (Table 1). Similar results were obtained by the study of preparations Nos. 4, 5, and 6; the action of the com-

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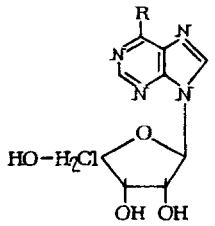
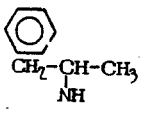
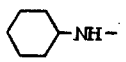
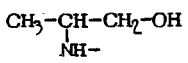
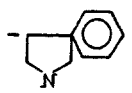
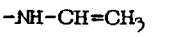
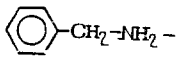
TABLE 1. N^6-Substituted Adenosine Derivatives. Binding with Purine Receptors in GPI and GPVD



Substance	R-	Molecular weight, daltons	ED_{50}, M	
			GPI	GPVD
PIA		371	$5,4 \cdot 10^{-11}$	—
CHA		349	$1,7 \cdot 10^{-10}$	$2,9 \cdot 10^{-4}$
Preparation 1		325	$2,9 \cdot 10^{-10}$	—
Preparation 2		349	$1,4 \cdot 10^{-3}$	—
Preparation 3		293	$5,2 \cdot 10^{-9}$	$3,1 \cdot 10^{-4}$
Preparation 4		335	$1,5 \cdot 10^{-3}$	—
Preparation 5		333	$1,7 \cdot 10^{-5}$	—
Preparation 6		357	$1,7 \cdot 10^{-5}$	—
ATP		507	—	$1,6 \cdot 10^{-6}$

pounds was not exhibited even when they were given in high concentrations (10^{-3}-10^{-5} M). It can thus be tentatively suggested that introduction of the substituents 3-phenylpyrrolidine, piperidinyl, morpholinyl, and benzyl into the purine ring makes these compounds unable to bind with purine receptors.

Meanwhile preparation 1 in a dose of $2.9 \cdot 10^{-9}$ M (R = 2-aminopropanol) inhibited contractility of GPI; the effect of this compound, moreover, was 1.7 times stronger than that of CHA but 18.6 times weaker than that of PIA. Preparation 3 (R = allylamine) had high affinity for A_1-receptors, but its effect was weaker than that of CHA and PIA.

Consequently, it can be postulated that preparations 1 and 3, CHA, and PIA possess a high degree of affinity for A_1-purine receptors of GPI, and that PIA has the strongest effect.

The question arises whether these adenosine derivatives can bind with A_2 receptors. Accordingly, in the subsequent experiments the effect of CHA and of preparation 3 (as among the most effective compounds relative to A_1 purine receptors, on contractility of GPVD was studied. Neither of these two preparations was found to cause contractions of GPVD, even in a concentration of 10^{-4} M, evidence of the virtually complete absence of affinity for A_2-purine receptors. For comparison, the concentration of ATP causing definite contraction of GPVD was $(1.6\text{-}2.4) \cdot 10^{-6}$ M.

The experimental results thus show that N^6-derivatives of adenosine have a high degree of affinity mainly for A_1-purine receptors, located in GPI. Introduction of allyl and 2-aminopropanol groups into the structure of the purine ring increases the affinity of the adenosine derivatives for purine receptors [4].

Meanwhile, the use of a model of the isolated GPI and GPVD allows the rapid screening of new drugs based on adenosine and having a selective action on the corresponding receptors, with the aim of creating a new class of neurotropic preparations.

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