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EFFECT OF NEW PYRIDOXAL PHOSPHATE ARYLAZO DERIVATIVES ON THE ECTO-ATPase ACTIVITY IN GUINEA PIG TISSUES

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EctoATPase (EC [3.6.1.3]) is a key enzyme in the enzymatic cascade of ATP hydrolysis to adenosine [1], There is convincing evidence that ectoATPase plays the role of an enzyme controlling the ATP interaction with P₂-purinoceptors [2], similar to the role of cholinesterase in the cholinergic synapse.

There are two types of ectoATPases, representing soluble ATPases secreted from neuron terminals [3] and insoluble ATPases. The latter enzymes belong to transmembrane proteins (glycoproteins) composed of several subunits [4], The enzymatic hydrolysis of ATP leads to the formation of adenosine – a highly active agonist of P₁-purinoceptors. In many cases, ATP and adenosine – affecting the P₂-and P₁-purinoceptors, respectively – may produce mutually compensating effects, thus complicating the interpretation of results. In addition, the effect of a pharmacologically-active agent upon ectoATPase may distort the real pattern of action of this substance upon the P₂-purinoceptors. In this context, substances capable of suppressing ATP metabolism would offer an important pharmacological means of elucidating the true effect of a given drug upon P₂-purinoceptors.

At present, there is an extensive search for selective inhibitors of ectoATPase activity. Various organic and inorganic compounds were reported to affect ectoATPase [5 – 7], but none of these showed good selectivity. As is known, most of the P₂-receptor antagonists are capable of suppressing ectoATPase [2, 8, 9]. By now, only one ectoATPase inhibitor described in the literature-ARL 67156 compound [10]-has proved not to affect P₂-purinoceptors, but this preparation is yet not commonly accessible.

Pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) is widely used as an effective P₂-purine receptor antagonist [2, 11, 12], but this compound is capable of suppressing the ectoATPase activity in various tissues [2, 9]. Previously [13, 14] we have established that some of the PPADS derivatives also exhibit antagonism with respect to P₂-purine receptors. The purpose of this work was to assess the effect of some new PPADS analogs on the ectoATPase activity.

METHODS OF INVESTIGATION

The experiments were performed with the muscle tissues 350 – 550 g of mongrel male guinea pigs killed by impact on the head and dehematization. The muscle tissue samples were prepared from urinary bladders, deferent ducts, and taeniae caeci as described in [13]. The ectoATPase activity was determined at $37 \pm 1^\circ\text{C}$ in a buffer containing 100 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES), 135 mM NaCl, 5 mM KCl, 2 mM CaCl_2 , 2 mM MgCl_2 , and 10 mM glucose (pH 7.4). Smooth muscle pieces (2–3 mg) were placed in a special 24-cell unit and washed for 15 min in the buffer to remove nucleotides and damaged cells. Then the washing buffer was removed and the samples were incubated for 10 ± 1 min in a buffer containing ATP at a concentration of 300 mM. To stop the enzymatic reaction, the buffer was removed from the cell and frozen. After that, the samples were washed again and incubated for 10 ± 1 min in the ATP-containing buffer to which the substance to be tested was added at a concentration of 10 μM .

The concentration of unhydrolyzed ATP was determined by HPLC. The analyses were performed using a Spherisorb (Hichrom, UK) column with an SPD-10A injector, linked to a LC-10AS spectrophotometer (Shimadzu, Japan). The column was preliminarily washed for 24 h with a mobile phase (0.2 M KH_2PO_4 + 3% methanol, pH 6.0) at a rate of 0.2 ml/min. The nucleotide separation was effected with the mobile phase flowing at a rate of 1.5 ml/min and detected at a wavelength of 260 nm using a recorder velocity of 1 cm/min; the sample volume was 20 μl . Under these experimental conditions, nucleotides were eluted in the following order: ATP, ADP, AMP. The ATP content was evaluated by comparing the corresponding peak heights for the sample and a standard ATP preparation. The enzyme activity was expressed in nmole/mg tissue/min. The effect of each substance studied was assessed by comparing the enzymatic reaction rates in the samples with and without the additive.

The experimental data were statistically processed by method of direct differences and presented in the $M \pm m$ form using the results of at least two independent measurements in four parallel runs.

ATP and HEPES were purchased from Sigma (USA). The PPADS analogs were synthesized at the laboratory of the National Institute of Diabetes and Diseases of the Alimentary System and Kidneys (Bethesda, USA). The chemical structures of these compounds are presented below.

Compound	X	R ¹	R ²	R ³	R ⁴
I	CH ₂ O	H	SO ₃ Na	H	H
II	CH ₂ O	H	COOH	H	H
III	CH ₂ O	H	H	COONa	H
IV	CH ₂ O	COONa	Cl	H	H
V	CH ₂ O	Cl	H	H	H
VI	CH ₂ O	Cl	H	H	SO ₃ Na
VII	CH ₂ O	H	Cl	COONa	H
VIII	CH ₂ O	Cl	H	H	NO ₂
IX	CH ₂	SO ₃ Na	H	H	SO ₃ Na
X	CH ₂	Cl	H	H	SO ₃ Na
XI	(CH ₂) ₂	SO ₃ Na	H	H	SO ₃ Na
XII	(CH ₂) ₂	Cl	H	H	SO ₃ Na
XIII	(CH ₂) ₂	H	H	COONa	H
XIV	CH=CH	SO ₃ Na	H	SO ₃ Na	H
XV	(CH ₂) ₃	SO ₃ Na	H	H	SO ₃ Na
XVI	(CH ₂) ₃	Cl	H	H	SO ₃ Na

Compound	X
XVII	CH ₃
XVIII	(CH ₂) ₃
XIX	CH=CH-CH ₂
XX	(N ₂) ₂
XXI	CH=CH

Compound	R ¹	R ²	R ³	R ⁴	R ⁵
XXII	H	SO ₃ Na	H	SO ₃ Na	H
XXIII	SO ₃ Na	H	SO ₃ Na	H	H
XXIV	H	COONa	H	H	Cl

RESULTS AND DISCUSSION

We have studied a total of 27 compounds. Reliable drug-induced changes in the ectoATPase activity were observed for 13 substances (Table 1). Most of these, producing various effects upon ectoATPase, also influenced P₂-purine receptors as was demonstrated previously [13, 14], while compounds I, V, XVII, and XXV selectively changed only the enzyme activity. Compound I, producing a 10% increase in the activity of ectoATPase in taenia caeci, inhibited P_{2Y}-purinoceptors in this tissue when taken at a greater concentration (30 μM) [13].

Compound V produced a 16% inhibition in the ectoATPase activity in taenia caeci, while compounds XVII and XXV inhibited the enzyme activity in the urinary bladder muscles (by 19 and 11%, respectively). However, these compounds cannot be used as ectoATPase inhibitors since the decrease in the enzyme activity never exceeds 20%. Moreover, compounds V and XVII (but not XXV) taken at a concentration of 30 μ M reliably inhibit P₂-purine receptors in both tissues (taenia caeci and urinary bladder) [13].

A special group includes nine compounds acting on both P₂-purine receptors and ectoATPase. The effect upon receptors does not allow these substances to be considered as selective ectoATPase inhibitors. The effect upon purine receptors is most probably also distorted by the action upon ectoATPase. Compounds V, IX, XVI, and XXI stimulate the ectoATPase activity in the muscles of deferent ducts, while compounds I, IV, XVII, XV, and XXI in the muscles of taenia caeci (Table 1). This may account for a pronounced antagonist effect observed in the pharmacological experiments with P₂-purine receptors. On the other hand, the ectoATPase inhibition observed for compounds IV, V, VIII, IX, XIII, XV, XVII, XI, and XXIV can probably mask their blocking action upon purine receptors in the corresponding tissues.

Of the 27 compounds studied, 14 exhibited no reliable action upon the ectoATPase activity in either of the tissues studied. It should be noted that compounds III and VII, previously characterized as promising selective antagonists of P₂-purine receptors [2, 13], produced no effect upon the enzyme. This result confirms our recommendations concerning the expediency of using these compounds as selective antagonists of P₂-purine receptors in physiological and pharmacological experiments *in vitro*.

Thus, the results of our experiments showed that 9 new PPADS derivatives exert a certain influence on the ectoATPase activity, which hinders correct interpretation of their effects upon P₂-purine receptors. It would be of interest to study the behavior of the ectoATPase activity under the action of some structural analogs of compound XXV, which may be promising inhibitors of ectoATPase. Compounds III and VII, previously characterized as selective antagonists of P₂-purine receptors, do not affect the ectoATPase activity.

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TABLE 1.

Effect of PPADS Derivatives at a Concentration of 10 μ M on the EctoATPase Activity in Isolated Tissues of Guinea Pigs ($M \pm m(n)$).

Com-pound	Tissue		
	deferent duct	urinary bladder	taenia caeci
I	98.1 $\pm$ 4.6 (8)	90.4 $\pm$ 4.8 (8)	110.3 $\pm$ 3.7 (8) *
IV	94.8 $\pm$ 4.4 (8)	85.5 $\pm$ 6.4 (8) *	118.1 $\pm$ 4.6(8) *
V	108.5 $\pm$ 2.0(8) *	107.2 $\pm$ 15.8 (8)	86.1 $\pm$ 4.4 (8) *
VIII	98.4 $\pm$ 7.6 (8)	84.7 $\pm$ 2.4 (8) *	90.0 $\pm$ 6.5 (8)
IX	123.7 $\pm$ 4.3 (8) *	104.9 $\pm$ 11.9 (8)	97.9 $\pm$ 3.7 (8)
XIII	89.4 $\pm$ 3.6 (8) *	101.4 $\pm$ 4.5 (8)	94.5 $\pm$ 6.6 (8)
XV	112.4 $\pm$ 14.1 (8)	101.3 $\pm$ 4.3 (8)	124.8 $\pm$ 8.6 (7) *
XVI	114.5 $\pm$ 6.7 (8) *	84.5 $\pm$ 3.6 (8)	115.8 $\pm$ 12.6(8)
XVII	120.5 $\pm$ 13.9(8)	81.4 $\pm$ 5.9 (8) *	129.7 $\pm$ 13.5 (8) *
XXI	116.8 $\pm$ 10.9(8)	90.0 $\pm$ 4.5 (8) *	92.3 $\pm$ 3.9 (8)
XXIII	121.8 $\pm$ 7.0 (8) *	97.5 $\pm$ 5.2 (8)	101.4 $\pm$ 12.5 (8)
XXIV	86.2 $\pm$ 5.6 (8) *	91.4 $\pm$ 4.6 (8)	114.6 $\pm$ 10.1 (8)
XXV	110.8 $\pm$ 10.8(8)	89.4 $\pm$ 2.7 (7) *	83.8 $\pm$ 5.0 (8) *

Note. All values are expressed relative to the initial ectoATPase activity in a given tissue taken as 100%.

* $p < 0.05$.