Antiviral Activity of Some Vasodilative Preparations

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, № 2, pp. 167-169, February, 1996
Original article submitted February 14, 1995

The effect of no-spa, papaverine, euphylline, and nitroglycerin on reproduction of influenza viruses A and B is studied in the chorioallantois of chick embryos and in cultured canine renal cells. The optimal effect was produced by no-spa, while euphylline was somewhat less effective. Neither papaverine nor nitroglycerin had any effects on reproduction of influenza viruses A and B. The antiviral activity of no-spa has also been proven in experiments with influenza infection in white mice.

Key Words: *vasodilative preparations; influenza viruses; reproduction*

Evidence appearing during the last ten years to the effect that many vasodilative preparations act via the formation of nitric oxide in the vascular endothelium [3,4,6] prompted us to study the possible antiviral effect of these preparations. Nitric oxide exhibits certain antimicrobial properties and is considered to be a factor which helps cells combat a microbial invasion [5,7]. However, many vasodilators have not yet been studied as potential antiviral agents.

At the outset of this study, we realized that the mere detection of antiviral activity of vasodilators would not prove the validity of our working assumption but would just be the first step on the road to future investigations. Nevertheless, we did discover such activity in some preparations and we report these data, leaving open the question as to whether nitric oxide is involved in the inhibition of virus reproduction.

MATERIALS AND METHODS

The following vasodilators were used: no-spa, papaverine, euphylline, and nitroglycerin.

Reproduction of the influenza virus was routinely studied using a culture of chick embryo chorioallantois (CEC) and in cultured canine renal cells (MDCK) as described elsewhere [1]. Influenza A virus strain A/Victoria/35/72 (H3N2) and influenza B virus strain B/USSR/100/85 were used as the model viruses. Antiviral activity was evaluated by measuring the infectious dose (ID), the concentration of preparation inhibiting reproduction of the viruses by 50% in comparison with the control (for the CEC culture) and by the hemagglutination titer in the culture medium (for the MDCK culture).

The efficiency of the vasodilators in experimental influenza was evaluated in white mice weighing 18-20 g. The animals were intranasally infected with influenza virus A/Aichi/2/68 (H3N2), which causes a 75-90% lethality. The protective effect of the preparations was assessed by the number of survivors. The protection index (PI) for the test preparations was calculated by the formula [2]: $PI=PC\text{-}1/PC\times100\%$, where *PC* (protection coefficient) is the ratio between the percentages of dead animals in the control and experimental groups.

A preparation is generally considered to possess antiviral activity if PI is equal to or exceeds 40.

RESULTS

Table 1 presents the data on the effect of the vasodilators on reproduction of the influenza viruses in the CEC culture.

It is seen that no-spa very effectively inhibited the reproduction of both type A virus (A/Victoria/35/72) and type B virus (B/USSR/100/85) by 3.0 log ID_{50} (for com-

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0007-4888/96/0002-0154$15.00

TABLE 1. Effect of Vasodilators on the Reproduction of Influenza Viruses in Cultured CEC Cells

Preparation	Dose, μg/ml	Drop in virus titer, log ID_{50}	
		A/Victoria/35/72	B/USSR/100/85
No-spa	25	3.0	3.0
Euphylline	48	2.0	1.5
Papaverine	10	none	none
Nitroglycerin	10	none	none

parison, remantadine, a specific antiinfluenza drug, suppresses the reproduction of type A influenza viruses by 3-4 log ID_{50}). Euphylline was less effective in inhibiting the reproduction of both A/Victoria/35/72 and B/USSR/100/85 (by 2 and 1.5 log ID_{50}, respectively). No antiviral activity was exhibited by papaverine and nitroglycerin. It should be emphasized that the concentrations of the preparations corresponded to those used in medical practice. The more pronounced inhibition of virus reproduction by no-spa corresponds to the more potent vasodilative effect of this preparation in comparison with euphylline and papaverine.

The antiviral activity of no-spa and euphylline was also evaluated by their effect on the reproduction of influenza viruses in the MDCK culture (Table 2).

No-spa completely inhibited hemagglutination activity (HA) of influenza virus A/Victoria/35/72 both 24 and 48 h after infection. Euphylline less markedly inhibited HA of A/Victoria/35/72 virus: after 24 hours the titer dropped from 1/16 (control) to 1/2, and after 48 hours from 1/64 (control) to 1/16. In the study of the reproduction of virus B/USSR/100/85 only no-spa inhibited HA, the titer being lowered from 1/128 to 1/4 postinjection. Euphylline did not affect the titer of HA of B/USSR/100/85 virus.

In *in vivo* experiments we used only the most active preparation no-spa.

The mice were infected with the virus in doses of 100, 10, and 1 LD_{50}, and no-spa was injected intraperitoneally in a dose of 20 mg/kg 1, 24, 48, 72, and 96 hours after infection. Table 3 shows that PI was 40, 50, and 63% for the corresponding doses, which indicates an antiviral activity of no-spa vis-a-vis influenza virus.

Thus, two of the studied vasodilators exhibit antiviral activity *in vitro*, and one of them, no-spa, also *in vivo*.

These facts by themselves are of both theoretical and practical importance. However, they are insufficient for drawing any conclusion on the mechanism of this phenomenon. Moreover, the absence of antiviral activity in nitroglycerin is at odds with our original hy-

TABLE 2. Effect of Vasodilators on the Reproduction of Influenza Viruses in Cultured MDCK Cells

Preparation	Dose, μg/ml	Time of reproduction, h	Titer of HA	
			A/Victoria/35/72	B/USSR/100/85
Control (no drugs)		24	1/16	0
		48	1/64	1/4
		72		1/128
No-spa	25	24	0	0
		48	0	0
		72		1/4
Euphylline	48	24	1/2	0
		48	1/16	1/4
		72		1/128

TABLE 3. Efficiency of No-Spa in Influenza Infection in White Mice (Influenza Virus A/Aichi/2/68 (H3N2)

LD_{50} of virus	Lethality				Protection index, %
	control		treatment		
	died/infected	%	died/infected	%	
100	10/10	100	9/15	60	40
10	10/10	100	5/10	50	50
1	9/13	69	3/12	25	63

pothesis on the role of nitric oxide produced by many vasodilators. Nevertheless, the marked protective effect of no-spa calls for further investigations of the antiviral activity of similar compounds.

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Effect of Mexidol on the Content of Transmitter Monoamines and Amino Acids in Rat Brain Structures

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, № 2, pp. 170-173, February, 1996
Original article submitted January 30, 1995

The content of two major classes of neurotransmitters (monoamines and amino acids) and their main metabolites is measured in rats at certain intervals after *per os* administration of mexidol (150 mg/kg). The level of dopamine and of its metabolite dihydroxyphenylacetic acid is found to be considerably elevated in the frontal cortex, suggesting a pronounced cortical component in the mechanism of action of mexidol.

Key Words: *mexidol; frontal cortex; dopamine; dihydroxyphenylacetic acid; γ-aminobutyric acid*

Mexidol (3-hydroxy-6-methyl-2-ethylpyridine succinate) is a water-soluble biogenic antioxidant, one of the 3-hydroxypyridine derivatives, that are structurally analogous to the compounds of the vitamin B_6 family. Experiments on rodents have demonstrated that mexidol (25-100 mg/kg) exhibits antihypoxic, antiamnestic, anxiolytic, antistress, and anticonvulsant effects, possesses heropsychotropic activity, and potentiates hexenal-induced sleep. A course of mexidol treatment is reported to result in a stable rearrangement of lipid-protein complexes of neuronal membranes in the rat brain [4]. However, the effect of mexidol on the brain neurotransmitter systems has not yet been studied.

There are ample data on the involvement of neurotransmitter systems in the mechanism of action of nootropics [1,9,13,14]. The monoaminergic and acidergic components of neural transmission are usually evaluated by measuring the content of various neurotransmitters in certain brain structures of laboratory animals. We believe that it is more useful to study the dynamics of these parameters, since quantitative changes in the content of neurotransmitters may not always be captured from measurements at some arbitrary chosen time intervals.

In light of this, the aim of the present study was to evaluate the content of two major classes of neu-

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0007-4888/96/0002-0156$15.00