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EFFECT OF CHOLINOLYTIC AND ADRENOBLOCKING AGENTS ON RESISTANCE OF RAT ERYTHROCYTES TO HYPOOSMOTIC HEMOLYSIS

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The effect of central cholinolytics and adrenoblockers on hemolysis of rat erythrocytes in hypoosmotic buffer was studied in vitro. At pH 7.4 and in a concentration of 10^{-4} M, hemolysis was prevented to the greatest degree by the central nicotinic (n) cholinolytics ethyldiphacil, diphacil[1], pediphen[2], tropacin[3], and the β-adrenoblocker propranolol. Erythrocytes were protected against hemolysis to a lesser degree by the central muscarinic (m) cholinolytics amizil[4] and glypin[5] and the α-adrenoblockers pyrroxan[6], sympatholytin[7], and phentolamine. The antihemolytic effect of the drugs reached a maximum in the course of 30 min and continued for several hours. A lower level of ionization of the drugs containing a tertiary nitrogen atom in their molecule was shown to correspond to greater protection of the erythrocytes against hemolysis. The prevention of hypoosmotic hemolysis is evidence of stabilization of the erythrocyte membrane by the substances studied. The possibility of stabilization of membrane formations not containing synaptic contacts must be taken into account when considering the mechanism of action of central n-cholinolytics and β-adrenoblockers.

KEY WORDS: central cholinolytics; adrenoblockers; erythrocytes; membranes; stabilization.

Evidence has now been obtained to show that not all effects of cholinolytics and adrenoblockers can be explained entirely by blockage of the postsynaptic receptors of the corresponding mediator system [1, 3, 6]. It is accordingly interesting to study interaction between such compounds and biological membranes not containing synaptic contacts. Erythrocytes are a widely used model for the study of the action of various compounds on membranes [5].

[1]Adiphenine; [2]1,1-diphenyl-5-diethylaminopentane; [3]2,3-dihydro-3-hydroxy-8-methylnortropidine diphenylacetate hydrochloride; [4]Benactyzine; [5]Unidentified; [6]Central α-adrenoblocker of USSR origin; [7]Dibenamine.

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TABLE 1. Effect of Central Cholinolytics and Adrenoblockers on Hypoosmoctic Hemolysis of Rat Erythrocytes (in % of control; M ± m)

Drug		Pharmacological properties	Concentration of drugs in incubation medium			
			10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M
Amizil		Central m-cholinolytic	63,3±6,2 (8)	77,4±4,0 (16)	92,9±2,9 (20)	113,2±14,5 (16)
	P		<0,002	<0,02	>0,05	>0,5
Glypin		The same	51,4±2,9 (24)	79,7±5,1 (12)	95,3±5,7 (8)	94,8±7,5 (12)
	P		<0,001	>0,2	>0,4	>0,5
Dipheridine		Central m- and n-cholinolytic	108,3±5,2 (4)	64,8±11 (12)	89,3±6,7 (24)	92,7±12,5 (12)
	P		>0,2	<0,025	>0,25	>0,5
Diphacil		Central n-cholinolytic	39,8±9,3 (8)	63,9±7,7 (16)	91,3±2,9 (19)	91,4±7,4 (14)
	P		<0,001	<0,005	<0,025	>0,4
Tropacin		The same	64,6±3,9 (28)	38,5±8,2 (12)	89,9±3,3 (8)	91,7±5,2 (16)
	P		<0,001	<0,005	<0,05	>0,3
Ethyldiphacil		" "	47,8±1,5 (8)	25,4±2,1 (8)	88,9±5,4 (15)	88,6±3,3 (8)
	P		<0,001	<0,001	>0,05	>0,05
Pediphen		" "	191,6±16,9 (12)	36,6±4,6 (28)	84,1±8,7 (44)	93,5±8,7 (24)
	P		<0,001	<0,001	>0,2	>0,5
Sympatholytin		α-Adrenoblocker	96,1±1,0 (4)	74,9±6,3 (8)	94,2±5,2 (8)	—
	P		<0,1	<0,01	>0,3	
Phentolamine		"	41,3±2,5 (4)	63,6±2,9 (8)	85,3±7,8 (12)	84,2±2,8 (4)
	P		<0,001	<0,001	>0,2	<0,02
Pyrroxan		Central α-adrenoblocker	48,4±4,0 (4)	81,4±6,8 (52)	100,8±6,1 (66)	105,5±4,1 (27)
	P		<0,002	<0,05	>0,9	>0,3
Propranolol		β-adrenoblocker	11,4±3,7 (4)	32,6±6,7 (12)	82,0±4,1 (16)	85,1±4,2 (4)
	P		<0,005	<0,001	<0,05	<0,05
Procaine		Local anesthetic	—	110,6±8,2 (8)	89,8±9,1 (8)	79,1±4,2 (4)
	P			>0,25	>0,3	<0,05
Trifluoperazine		Neuroleptic	140,0±0,9 (4)	37,5±13,9 (20)	28,5±3,5 (28)	65,0±5,2 (4)
	P		<0,001	<0,002	<0,001	<0,02
Amphetamine		Adrenomimetic	106,9±2,0 (8)	95,3±5,9 (12)	95,3±2,1 (24)	97,6±2,6 (12)
	P		>0,1	>0,5	>0,1	>0,4

Legend. Here and in Table 2, number of observations given in parentheses.

In the present investigation the effect of central m- and n-cholinolytics, α- and β-adrenoblockers, and certain other substances (trifluoperazine, amphetamine, procaine) on the resistance of rat erythrocytes to hypoosmotic hemolysis was studied.

EXPERIMENTAL METHOD

In experiments to study the effect of the concentration of the drugs on hemolysis of erythrocytes, 0.05 ml of blood obtained from albino rats was added to 5 ml of 5 mM sodium-phosphate buffer, pH 7.4, containing 0.425% NaCl and 0.2 ml of an aqueous solution of the drug (experimental samples) or 0.2 ml of water (control samples). After careful mixing the samples were incubated for 5 min at room temperature and centrifuged at 4000 rpm. The optical density of the supernatant was determined by the SF-4 spectrophotometer at a wavelength of 540 nm. The extinction characterized the outflow of hemoglobin from the erythrocytes and was directly proportional to the degree of hemolysis. In experiments in which the pH of the incubation medium was varied under the same experimental conditions a 5 mM sodium phosphate buffer containing 0.425% NaCl, pH 6.6 and 7.75, was used. In the experiments to study the relationship between the antihemolytic effect of the drugs and time, control and experimental samples were incubated in a waterbath at 37°C with careful mixing for the required period.

EXPERIMENTAL RESULTS

Central cholinolytics and adrenoblockers, used in concentrations of 10^{-4} M, protected the erythrocytes against hemolysis in a hypotonic sodium phosphate buffer. The strongest protective action was exhibited by the central n-cholinolytics (ethyldiphacil, pediphen, tropacin, and diphacil) and the β-adrenoblocker propranolol.

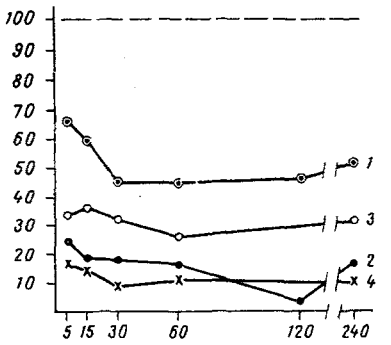

Fig. 1. Effect of central cholinolytics and adrenoblockers on hypoosmotic hemolysis of rat erythrocytes (in % of control) depending on duration of incubation. 1) Glycine 10^{-4} M; 2) tropacin 10^{-4} M; 3) pyrroxan 10^{-4} M; 4) propranolol 10^{-4} M. All values shown on graph differ significantly from control ($P < 0.05$). Abscissa, time (in min); ordinate, hemolysis (in % of control).

The central m-cholinolytics – amizil, glypin, and the α-adrenoblockers – pyrroxan, sympatholytin, and phentolamine, were less effective (Table 1). Potentiation of the antihemolytic action with an increase in the concentration of the drugs from 10^{-6} to 10^{-4} M indicates that within this dose range the intensity of the protective effect depended on the number of molecules of cholinolytic or adrenoblocker interacting with the erythrocyte membrane. In a concentration of 10^{-3} M the antihemolytic effect of many of the compounds was increased, but pediphen and dipheridine, and also the neuroleptic trifluoroperazine, in this dose, potentiated hemolysis. The antihemolytic action of ethyldiphacil, tropacin, and sympatholytin in a concentration of 10^{-3} M was weaker than in a concentration of 10^{-4} M (Table 1). The appearance of a hemolytic effect of certain drugs in high concentrations was observed by Seeman and Weinstein [8] and, in their opinion, it is due to irreversible lytic injuries to the erythrocyte membrane.

The relationship between the resistance of the erythrocytes to hypoosmotic hemolysis and the duration of action of the drugs on the erythrocyte membrane was studied for the cholinolytics glypin and tropacin and the adrenoblockers pyrroxan and propranolol. The antihemolytic effect of all four drugs was found to reach a maximum 30 min after the beginning of incubation, and it persisted for 4 h (maximal duration of incubation in these experiments). Just as in the experiments to study the dependence of the antihemolytic action of the drugs on its concentration, the n-cholinolytic tropacin inhibited hemolysis much more strongly than the m-cholinolytic glypin; the same was also true of the β-adrenoblocker propranolol compared with the α-adrenoblocker pyrroxan (Fig. 1). These results point to gradual saturation of the erythrocyte membrane by molecules of the drugs and that binding of the drugs with the membrane components takes place for a relatively long time, at least several hours.

Considering that the drugs studied contain a tertiary nitrogen atom in their structure and, since they are weak organic bases which are ionized to different degrees at pH 7.4, the next step was to study to what extent the degree of ionization affects the antihemolytic action of some of them. At pH 7.75, a lower degree of ionization of the drugs was shown to correspond to greater protection of the erythrocytes against hypoosmotic hemolysis than at pH 6.6, when they were practically completely ionized (Table 2). These results indicate that the hydrophobic regions of the erythrocyte membrane are optimal for binding drugs containing a tertiary nitrogen atom in their structure.

Prevention of hypoosmotic hemolysis reflects stabilization of the erythrocyte membrane and has been demonstrated for a wide range of local anesthetics [7]. The results indicate that a large group of central cholinolytics and adrenoblockers containing an amino group in their structure has this effect. However, other components of the molecule of the blockers play an important role in the stabilizing effect, for the adrenomimetic amphetamine, which also contains an amino group, did not protect the erythrocytes against hemolysis (Table 1). The use of erythrocytes as a model for studying action on membranes confirms the opinion of Professor S. S. Krylov [2] that central n-cholinolytics can stabilize membranes, including those which do not contain postsynaptic structures. Central n-cholinolytics are known to have a marked local anesthetic action [4]. Stabilization of membranes of nerve endings in the CNS by compounds of this sort may prevent release of neurotransmitters and thereby abolish their action on postsynaptic receptors. In all probability, an essential

TABLE 2. Effect of Central Cholinolytics and Adrenoblockers in Concentration of 10^{-4} M on Hypoosmotic Hemolysis of Rat Erythrocytes (in % of control) at Different pH Values of Incubation Medium (M ± m)

Drug	pH of incubation medium			
	6,6		7,75	
Amizil	97,4±4,0 (8)		76,7±8,3 (8)	
P		>0,5		>0,05
Glypin	95,4±2,7 (12)		47,3±3,1 (8)	
P		>0,2		<0,001
Diphacil	94,3±3,2 (8)		50,3±6,4 (8)	
P		>0,25		<0,001
Tropacin	88,4±3,3 (12)		8,5±2,2 (8)	
P		<0,02		<0,001
Ethyldiphacil	96,0±1,9 (4)		5,4±1,9 (4)	
P		>0,05		<0,001
Sympatholytin	101,4±3,8 (4)		35,9±2,6 (4)	
P		>0,7		<0,001
Pyrroxan	98,5±4,1		35,9±3,1	
P		>0,7		<0,001

feature of the mechanism of action of the β-adrenoblocker propranolol is its ability to prevent the release of catecholamines from nerve endings in the CNS and in organs receiving a sympathetic innervation. The weaker protection of erythrocytes against hypoosmotic hemolysis when central m-cholinolytics and α-adrenoblockers were used suggests that stabilization of the membranes plays a less important role in the mechanism of action of these compounds. Meanwhile the presence of glypin and pyrroxan in biological membranes for several hours, as shown by the antihemolytic test, points to the possibility that the properties of these membranes may be modified under the influence of these drugs.

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