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A NEW MOLECULAR MECHANISM OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR BLOCKADE

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KEY WORDS: nicotinic acetylcholine receptor; ionic channel; restorative properties; reducing properties; disulfide bonds

Three basic mechanisms of blocking of neuronal nicotinic acetylcholine (N-Ach) receptors have been described: blocking the open ionic channel, blocking the recognition center (competitive type of blockade), and blocking the closed ionic channel [4]. However, the molecular mechanism proper of ionic channel function has not been adequately studied. We attempted to link this mechanism with oxidation—reduction changes in sulfhydryl and disulfide groups appearing in the N-Ach receptor, and blockade of the receptor with reduction of S—S bonds in its active center.

The basis for the present investigation was, first, that previously we found marked oxidation—reduction properties in central nicotinic cholinolytics, compounds belonging to different chemical groups [3, 6], and second, data in the literature indicating that a functionally important disulfide bond is present in the active center of the N-Ach receptor, and its reduction leads to blockade of the receptor, whereas oxidation abolishes this blocking effect [7, 9].

EXPERIMENTAL METHOD

The central nicotinic cholinolytic activity was assessed in the nicotine test on albino mice and rats [1, 8]. Indices of protection were calculated by computer by probit analysis. Contractions of a segment of isolated rat ileum were recorded under isometric conditions by a 6M × 9B mechanotron [2]. The experimental results were analyzed by comparing mean values of EC_{50} obtained by logit analysis. The concentration of SH-groups was determined in the supernatant of rat brain (3000 × 15 min) spectrophotometrically in medium of the following composition: 50 mM Tris-HCl (pH 7.45), 20 mM KCl, 100 mM NaCl, 60 mM $MgCl_2$, 30 mM ATP. The volume of the sample was 1 ml. Samples were incubated for 30 min at

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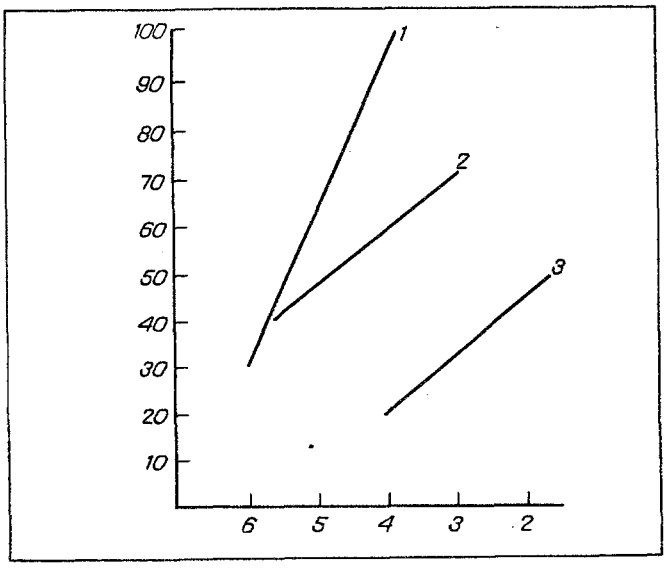

Fig. 1. Bolograms of concentration — effect logarithms for nicotine (isolated segment of rat ileum). Abscissa, negative logarithm of nicotine concentration; ordinate, effect (in % of maximal). 1) Nicotine without nicotinic cholinolytic, 2) nicotine + nicotinic cholinolytic pediphen (0.1 μM), 3) nicotine + ionol (10 μM).

37°C with constant shaking. The nicotinic cholinolytics for testing were dissolved in Tris-HCl and ionol was added to the samples in powder form.

EXPERIMENTAL RESULTS

The writers showed previously that nicotinic cholinolytics (altogether more than 25 compounds were tested) possess marked antioxidative activity [3, 6]. Depending on the experimental conditions, they exhibit either reducing or oxidizing properties. We suggested that the reducing properties of nicotinic cholinolytics are connected with the molecular mechanisms of blockade of neuronal N-Ach receptors. If these properties of nicotinic cholinolytics are responsible for blockade of neuronal N-Ach receptors, reducing disulfide bonds in the receptor protein molecule, known antioxidants and agents reducing disulfide bonds ought to possess nicotinic cholinolytic properties. This hypothesis was fully confirmed: ionol, like the central nicotinic cholinolytic pediphen, prevented contraction of the isolated rat intestine induced by nicotine (Fig. 1). The blocking effect of ionol is evidently specific and is realized through the N-Ach receptor. This is indicated by the fact that ionol does not prevent contractions of the isolated segment of intestine developing as a result of potassium depolarization. The magnitude of contraction induced by KCl was 100 ± 2.1%, compared with 96.8 ± 2.0% for ionol (10 μM).

Ionol and sodium diethyldithiocarbamate (DDC), a reducing agent of disulfide bonds, also possess central nicotinic cholinolytic activity (Table 1). The antioxidant ionol prevented development of nicotine tremor and seizures in albino mice in therapeutic doses, evidence of the specificity of its action.

The index of protection for DDC in a dose of 100 mg/kg, relative to prevention of the production of tremor and convulsions by the action of nicotine in the mice was 1.76 ± 0.71 and 1.35 ± 0.25 respectively, whereas for preventing death of the mice from nicotine it was 2.30 ± 0.50 ($p < 0.01$).

Like ionol, pediphen reduces disulfide bonds in brain supernatant (Table 2); these compounds, moreover, exhibit their activity only in the presence of Na^+, K^+, and Mg^{2+} ions. Pediphen also has the same effect on synaptosomes isolated from rat cerebral cortex.

TABLE 1. Central Nicotinic Cholinolytic Activity of Pediphen and Ionol (rats; M ± m)

Dose, mg/kg	Index of protection			
	pediphen		ionol	
	tremor	convulsion	tremor	convulsions
15	1,54±0,42	1,37±0,33		
25	2,18±0,53*	1,92±0,42*	1,30±0,33	1,37±0,35
50			1,42±0,40	1,25±0,36
100			2,37±0,56*	2,22±0,51*

Legend. *$p < 0.01$

TABLE 2. Effect of Pediphen and Ionol on Concentration of SH-Groups in Mouse Brain Supernatant (in mmoles/ml supernatant; M ± m)

Control	Pediphen		Ionol	
	0,1	0,01	0,001	1
0,718±0,013	0,809±0,017**	0,813±0,26*	0,793±0,42*	0,873±0,23***

Legend. *$p < 0.05$, **$p < 0.02$, ***$p < 0.01$.

TABLE 3. Effect of Pediphen (in %) on Contractile Activity of Isolated Rat Ileum, Induced by Nicotine in a Dose of 0.1 mM (M ± m)

Control	Amplitude of contractions		
	blockade by pediphen (1 μM)	rinsing with physiological saline (30 min)	rinsing with DTNBA (30 min)
100	50±3	54±4	97±14*

Legend. *$p < 0.01$ compared with value after rinsing with physiological saline.

The method of chemical modification, usually used to study receptor structure, was used in the present experiments to study interaction of the specific ligand of the nicotinic cholinolytic with the corresponding receptor at the molecular level. When this method was used, it was shown that rinsing the preparation of ileum with physiological saline did not abolish spasm induced by the nicotinic cholinolytic pediphen, whereas addition of 5,5'-dithio-bis-2-nitrobenzoic acid (DTNBA), an agent oxidizing sulfhydryl groups, to the solution led to complete restoration of contractility of the preparation of isolated ileum (Table 3).

The experimental results suggest that blockade of the neuronal N-Ach receptor takes place as a result of reduction of disulfide bonds in its protein molecule, and that the reducing agent of this bond is the nicotinic cholinolytic itself. Typical nicotinic cholinolytics evidently differ from reducing agents in possessing high affinity for the N-Ach receptor, and it is this which determines the specificity of their interaction with the receptor, whereas direct blockade of the ionic channel takes place as a result of the reducing properties of these compounds.

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EFFECT OF DIFFERENT PROTEINS ON PROTEOGLYCAN INDUCED ERYTHROCYTE AGGREGATION

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The ability of proteoglycans to exclude cells, cellular organelles, and compartments, and also other structural elements of tissues from the space occupied by them, to concentrate all this in a limited volume, and to prevent their dispersion has been studied in considerable detail in model experiments and on tissue cultures [3-7, 10-13]. However, in none of these investigations has the effect of the protein components of the medium on steric exclusion and aggregation of cells induced by proteoglycans been studied. The aim of the present investigation was to study effects of protein components of the blood plasma on steric exclusion and aggregation of erythrocytes in their suspension in salt solution, used as a model of isolated cells, realized by hyaluronic acid (HUA) and a natural protein — chondroitin — keratan sulfate (PCKS) complex. The need for such research is dictated by the fact that proteoglycans act in the tissues as steric exclusion factors always in the presence of various proteins.

EXPERIMENTAL METHOD

An equilibrium suspension containing 30% of rabbit erythrocytes by volume was made up from erythrocytes washed free from blood plasma with salt solution (0.15 M NaCl, pH 7.2, phosphate buffer) in the same solution. In experiments with whole blood, stabilized by citrate, the volume of the erythrocytes also was made up to 30%. Quantitative determination of erythrocyte aggregation was carried out by measuring the rate of separation of the suspension and blood into phases of solution (or plasma) and blood cells at 20°C [3]. The initial rate of this separation was determined as the tangent of the angle of slope of the curve to the abscissa. Highly purified and high-polymer preparations of HUA and PCKS were obtained in the form of the normal potassium salts, by methods suggested by ourselves [1, 2]. A total fraction of albumins, ($\alpha + \beta$)-globulins, and γ-globulin were isolated from rabbit serum (the same serum as that from which the erythrocytes

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