INHIBITION OF ACTIVE H^+ TRANSPORT BY LOCAL ANESTHETICS AND BARBITURATES IN MEMBRANES OF BRAIN SYNAPTIC VESICLES

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An acid medium (pH about 5.0) is maintained in the synaptic vesicles (SV), and is essential for processes such as accumulation of neurotransmitters and their precursors, their metabolism, and proteolytic processing of neuropeptides to take place [10]. Maintenance of the acid medium (pH gradient) in SV is an active process, involving the participation of the ATP-dependent H^+-pump [3], consisting of H^+-ATPase [4]. Generation of ΔpH takes place only in the presence of the penetrating Cl^- anion, by combined working of the electrogenic H^+-pump and the anionic (chloride) channel [2]. Topologic relations between the H^+-pump and the anionic channel are unknown, but it is considered that the link between them is indirect and purely functional in character [2].

It is not only the mechanism of coupling between the H^+-pump and the anionic channel that is unknown, but the whole range of their functions in the SV membrane has by no means been fully explained. The pharmacologic characteristics of both components of this coupled system are unavailable, and the specific inhibitors which would enable each component to be selectively and separately influenced are virtually unknown. The writers showed previously that classical convulsants, known to be ligands of the GABA-receptor/chloride channel complex, inhibit active H^+ transport in the membranes of CV, and moreover, the mechanism of their action is blockade of the anionic channel [5]. The strongest effect is given by bicuculline, followed by picrotoxin, penicillin, bemegride, and metrazol [5].

In continuing this work we directed our attention to two groups of compounds which are related to the action of convulsants and, in the wider sense, to epileptic activity (EA). Lcoal anesthetics, when administered systemically, depending on their concentration, may have an antiepileptic action, but they may also induce generalized EA [8]. Barbiturates, with their well-known anesthetic properties, are known as activators of the GABA-receptor complex [9, 12], and some of them are used in the treatment of epilepsy [9, 12].

EXPERIMENTAL METHOD

Membranes of SV, without their contents, were obtained from rat brain by the method described previously [4]. The residue of SV was suspended in 0.32 M sucrose, 10 mM HEPES/Tris, pH 7.4, and kept at —20°C for 1-2 weeks.

ATP-dependent H^+ transport as a measure of activity of the H^+-pump and of the anionic channel coupled with it was measured by means of the fluorescent probe acridine orange (AO), as described previously [4]. The measuring medium contained 2 μM AO in 150 mM KCl, 20 mM HEPES/Tris, pH 7.4, at 25°C. The initial velocity of H^+ transport and total accumulation of protons were calculated from the curves of quenching of fluorescence of the probe after addition of Mg-ATP [4].

Local anesthetics of Soviet manufacture were used: procaine, lidocaine, trimecaine, and tetracaine, in the form of aqueous solutions. The following barbiturates of Soviet manufacture were used: barbital and phenobarbital in the form of the free acids, dissolving them with alkalification by Tris base; barbital-sodium, amobarbital, pentobarbital, and hexobarbital in the form of the sodium salts; their solutions, with pH 9.0-10.0, were neutralized by HCl; benzobarbital was dissolved in dimethylsulfoxide.

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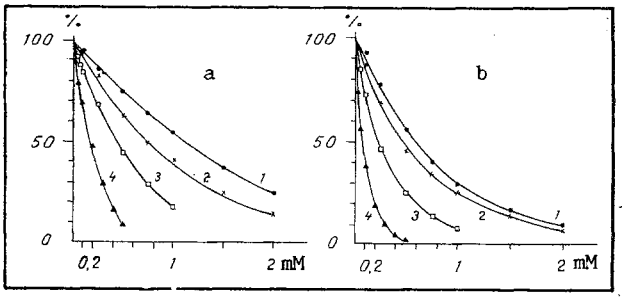

Fig. 1. Dependence of inhibition by local anesthetics of ATP-dependent H^+ transport in SV membranes on concentration. 1) Procaine, 2) lidocaine, 3) trimecaine, 4) tetracaine. Here and in Fig. 2: abscissa, concentrations of drugs; ordinate, initial velocity of H^+ transport (a) and total accumulation of protons (b).

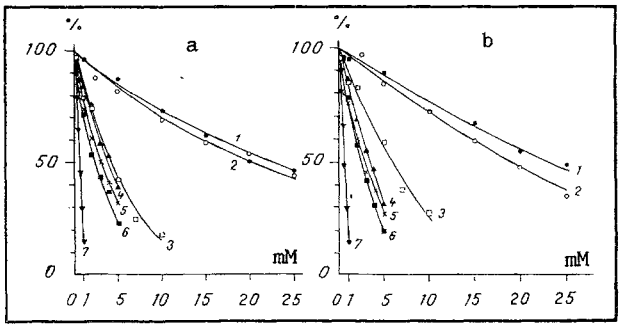

Fig. 2. Dependence of inhibition of active H^+ transport in SV membranes by barbiturates on concentration. 1) Barbital sodium, 2) barbital, 3) phenobarbital, 4) pentobarbital, 5) amobarbital, 6) hexobarbital, 7) benzobarbital.

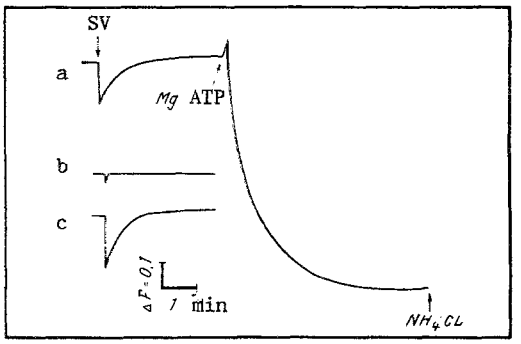

Fig. 3. Changes in fluorescence of AO in response to successive addition of SV membranes and Mg-ATP. a) In standard measuring medium, b) in the presence of 0.5 mM tetracaine, c) in the presence of 10 mM phenobarbital.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that all the local anesthetics inhibited H^+ transport, and the inhibition was concentration-dependent. Both parameters, namely initial velocity and total accumulation of H^+, changed virtually identically. As regards the effectiveness of inhibition the local anesthetics could be arranged in the same order as is known by their anesthetic action [8], namely: tetracaine > trimecaine > lidocaine > procaine. Values of IC_{50} (concentration causing half of the maximal inhibition) were 0.07, 0.21, 0.48, and 0.60 mM respectively.

Barbiturates inhibited ATP-dependent H^+ transport in higher concentrations (Fig. 2). The example of barbital shows that inhibition was virtually independent of the form (free acid or sodium salt) in which the barbiturates were used. Benzobarbital had the strongest action, barbital the weakest; their IC_{50} values differed by 40 times (0.5 and 20 mM respectively). The remaining barbiturates differed less from one another, and formed a quite homogeneous group, in which the values of IC_{50} lay within the range 2-5 mM. As regards the effectiveness of their inhibition the barbiturates were arranged in the following order: benzobarbital $\gg$ hexobarbital > amobarbital > pentobarbital > phenobarbital $\gg$ barbital.

Unlike the local anesthetics, which abolished not only the ATP-dependent, but also the endogenous pH gradient, which was partly preserved in SV after their isolation, and dissipated spontaneously in the cuvette in the absence of ATP, barbiturates had no effect on the endogenous pH gradient. This is clear from the fact that in the presence of barbiturate the reaction of the probe to addition of SV still remained, whereas in the presence of the local anesthetic it disappeared (Fig. 3). In this respect local anesthetics behave like typical weak bases, which indeed they are.

Inhibition of active H^+ transport in SV membranes is a new and hitherto unknown property of local anesthetics and barbiturates. They considerably widen the list of drugs capable of influencing maintenance of the pH gradient in brain SV, which was previously limited to the classical convulsants [5] and trifluoperazine, known to be a calmodulin antagonist [1]. Both groups — local anesthetics and barbiturates — give rise to multiple effects in vivo and in vitro, and it is therefore essential to analyze which of them correlate with the inhibition of H^+ transport found in SV.

As regards local anesthetics, first of all no correlation was found with their antiepileptic action. Concentrations in which inhibition of H^+ transport is observed were closer to those noted in the blood during toxic manifestation, i.e., generalized convulsions [8]. The mechanism of the convulsant action of local anesthetics is not clear. The order of effectiveness in which the local anesthetics can be arranged for inhibition of H^+ transport coincides with that characteristic for both their anesthetic and their convulsant action [8]. In all cases the same structural factors evidently play a role, and only the targets and their affinity for local anesthetics may be different.

It follows from Fig. 3 that with respect to the mechanism of their action on H^+ transport local anesthetics are similar to trifluoperazine [1]: being penetrating weak bases they act as uncouplers, by-passing the H^+-pump. Admittedly it is impossible to rule out completely the possibility of interaction with the H^+-pump or anionic channel, for interaction of this kind will be masked by the uncoupling effect. Whether this mechanism is related to the convulsant action is not clear. Whatever the case, the ability of another weak base (ammonia) to induce convulsions and EA is well known [7].

In relation to barbiturates, correlation with the antiepileptic action likewise is not observed. Clinically effective compounds, namely phenobarbital and benzobarbital, differ appreciably in their ability to inhibit H^+ transport. Values of IC_{50} in fact differ by an order of magnitude (Fig. 2). On the other hand, convulsant properties in general are uncharacteristic of the barbiturates which we studied. Marked correlation was observed only with the anesthetic action of the barbiturates: the order of their effectiveness is the same [9, 12].

Barbiturates had no effect on the endogenous pH gradient (Fig. 3), which means that they have no uncoupling effects. Consequently, inhibition of H^+ transport may be caused by interaction either with the H^+-pump or with the anionic channel. At physiological pH values barbiturates, being weak acids, are at least half in solution in the form of anions, and it therefore seems likely that the anionic channel may be blocked by a competitive mechanism. An analogy can be drawn with the GABA-dependent Cl^--channel, in relation to which barbiturates have recently been shown to have a biphasic action: activation with an increase in concentration, well known in the literature [9, 12], gives way to deep inhibition [6, 11]. The concentration range in which inhibition of GABA-dependent responses is observed is close to that in which barbiturates inhibit H^+ transport in SV membranes. The possibility cannot be ruled out that the ability of barbiturates (in high concentrations) to act as blockers may be one further proof of the structural similarity of the different anionic channels.

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EFFECT OF LOW-INTENSITY LASER IRRADIATION ON STATE OF BLOOD PROTEINS

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Low-intensity laser radiation is widely used at the present ime in medicine for the treatment of various diseases [1,6-9,11,13,14]. However, the mechanism of its action is not yet clear. This is largely due to the fact that most investigators have studied the response of the whole body to irradiation. There have been few studies in vitro. For instance, the effect of irradiation has been studied on platelet and leukocyte function, and on the state of the blood clotting system and enzyme activity [3-7, 10, 12]. Even these parameters, however, are essentially a reflection of a series of complex processes taking place in the blood and its components under the influence of irradiation. Simpler parameters reflecting the response to irradiation in vitro are required. In the investigation described below the degree of binding of a fluorescent probe by blood proteins and cells was chosen as the parameter.

EXPERIMENTAL METHOD

Whole blood, plasma, and platelet mass. The effect of irradiation was assessed with the aid of the fluorescent probes 1,8-anilinonaphthalene sulfonate (ANS) and 4-(p-dimethylaminostyryl)-1-methylpyridinium (DSM). Fluorescence of ANS was excited at a wavelength of 270 nm and recorded within a wide range of wavelengths in excess of 360 nm. The ranges for DSM were 470 and 530 nm respectively. All investigations were carried out on the "Specord M40" spectrophotometer.

Whole bood was irradiated in a Petri dish in a thin layer (1-2 mm) by the unfocused beam of a helium-neon laser (wavelength 632.8 nm), with power density of 0.9 mW/cm^2 for 5-60 min. The irradiated blood was then incubated under room conditions in darkness for between 10 min and 24 h, after which the blood cells were separated by centrifugation at 1500 rpm for 15 min. The supernatant was diluted with 0.9% NaCl solution in a ratio of 1:50 by volume. To 2 ml of the resulting solution 0.01 ml of 10^{-4} M ANS in water was added. The optical density of the plasma and probe were verified photometrically at 270 nm. Platelet mass or plasma was irradiated in a test tube by a laser beam with power density of 45 mW/cm^2. The difference in power density for irradiation of blood compared with irradiation of platelet mass and plasma is due to the necessity of irradiating the blood with an unfocused laser beam in a thin layer.

All the materials studied (blood, plasma, platelet mass) were divided into two series: control and experimental; the difference between them was limited to the irradiation procedure.

The "effective binding constant" K_{ef} was chosen as the quantitative characteristic reflecting the results:

$$K_{ef} = \frac{I}{D_1 (D_2 - D_1)},$$

where I denotes the intensity of fluorescence in the selected spectral range in arbitrary units, D_1 the optical density (in relative units) of the test sample at 270 nm without the probe, and D_2 the optical density of the same sample with the probe. The value of $D_2 - D_1$ is evidently proportional to the concentration of the probe, whereas D_1, even allowing for the contribution of scatter to the optical density, is basically proportional to the protein concentration for this particular

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