Kinetics of Interaction of Local Anesthetics with Human Serum Albumin

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We studied kinetic parameters of interaction of local anesthetics (lidocaine, tetracaine, bupivacaine, and two novel agents with proved local anesthetic potency RU-353 and RU-1117) with human serum albumin. Complexation of local anesthetics with human serum albumin is a time-dependent and reversible process; equilibrium was attained within 1.5-4.5 h depending on chemical nature of local anesthetics.

Key Words: *albumin; local anesthetics; interaction kinetics*

Evaluation of mechanisms of interaction of local anesthetics (LA) with transport systems in the blood makes it possible to predict their pharmacokinetic and pharmacodynamic properties: the degree and duration of anesthetizing action, the rate of metabolism and/or utilization in peripheral tissues [7].

Physicochemical studies showed that human serum albumin (HSA) has several binding sites involved in specific and stereospecific interaction with various ligands. By their selectivity, these sites are similar to neurotransmitter and hormone recognition sites on receptors. The HSA molecule has several domains of high binding ability with association constant of 10^8-10^6 M^{-1}, including binding sites for fatty acids, bilirubin, indole, and warfarin. Typical binding site consists of two compartments: a hydrophobic "pocket" (cavity) formed by radicals of nonpolar amino acid residues and cationic center located in this cavity or near it. A high degree of complexation with HAS is typical of low-molecular drugs characterized by week acid properties. In this case, stoichiometry of the albumin—ligand complex and the total binding capacity are 1:2 and 1.2 mmol/liter, respectively [10].

Experimental and clinical studies showed that an "incubation period" is required for HAS-based LA solutions: anesthetic efficiency depends on the duration of preincubation of LA with HAS before their injection *in vivo* [3]. Kinetic parameters of HAS—LA system were not measured, although some estimates were made.

Our aim was to study the kinetics of the interaction of HAS with well-known LA and novel promising compounds with established anesthetic potency.

MATERIALS AND METHODS

Tritium-labeled LA were obtained by the standard method by labeling of tertiary amines [4]. Specific activity of these preparations was 54±10 Ci/mol.

Kinetic parameters of interaction of HAS and ^{3}H-LA (direct effect) and LA-binding activity of HAS in the presence of an excess of cold LA (reversibility) were evaluated under standard conditions (0.05 M sodium phosphate buffer, pH 7.4, 22±1°C) using dextran-coated charcoal adsorption of free ligand with correction for background binding (in the absence of HAS) [2]. The concentrations of HAS and LA were chosen in such a way, that only 40-50% of labeled LA was bound.

The results were analyzed statistically using Student's *t* test.

RESULTS

We previously examined the equilibrium parameters of HAS—LA interaction using 1-anilino-8-naphtha-

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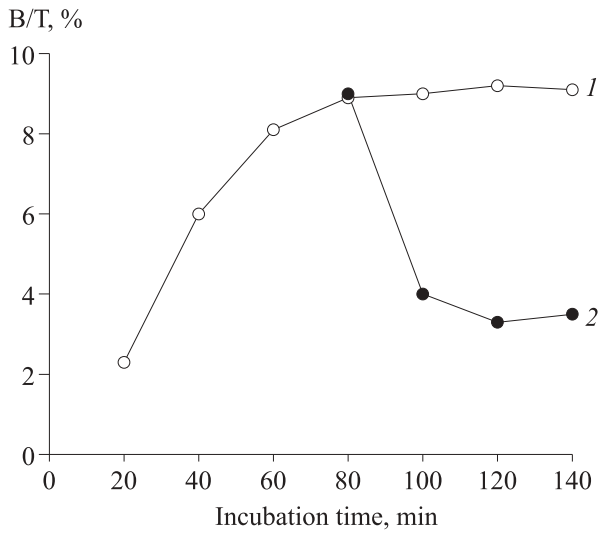

Fig. 1. Kinetics of ^{3}H-lidocaine complexation with HAS and dissociation of the complex at 22°C. *1*) percent of binding (B/T) as a function of incubation time; *2*) replacement of labeled lidocaine in HAS complex with nonlabeled molecules.

TABLE 1. Kinetic Parameters of HAS and LA Complexation (*M*±*m*)

Preparation	Time to equilibrium, h	Half-dissociation time, min
Lidocaine	0.5±0.1	19±2
RU-353	2.4±0.2	20±4
RU-1117	3.6±0.3	26±5
Tetracaine	4.5±0.5	15±4
Bupivacaine	4.0±0.4	28±5

Note. The mean values were obtained in 3 independent experiments for each LA.

lene sulfonate (ANS) as a fluorescent probe [1]. Complexation of LA with HAS decreased in the following order: bupivacaine>RU-1117>tetracaine>>lidocaine> RU-353>procaine. This method is based on changes in ANS fluorescence caused by changes in local charge, rigidity, or conformation.

However, the use of fluorescent probes for evaluation of kinetic parameters of HAS—LA interaction is impossible [6,8]. To this end, the radioligand methods are most frequently used, which quantitatively assess binding of a ^{3}H-, ^{14}C-, or ^{125}I-labeled compound. Labeling LA with ^{14}C is possible only at the stage of its chemical synthesis, so application of ^{14}C-ligands ensures best reproducibility of the experimental data. However, this method is most expensive. In contrast, iodine isotope enhances sensitivity of the radioligand essay, although it can modulate complexation properties of the ligand [5]. In this study, we modified LA with radioactive tritium.

The time-dependence and reversibility of complexation of labeled LA with HAS were examined for all test LA. Typical kinetic curves are exemplified for lidocaine (Fig. 1, curve *1*). Abscissa corresponds to the total incubation time at 22°C in the cold. In our experiments we varied only the time of warm incubation, while subsequent cooling of the system for stabilization of the complex before charcoal adsorption always lasted 10 min. Introduction of cold lidocaine excess after attaining the equilibrium resulted in partial dissociation of the ^{3}H-lidocaine—HAS complex (Fig. 1, curve *2*). The kinetic parameters of HAS complexation with different LA are shown in Table 1.

The time to equilibrium significantly differed depending on chemical nature of LA: it was minimum for lidocaine (1.5 h) and maximum for tetracaine (4.5 h) and bupivacaine (4.0 h). The novel agents RU-353 and RU-1117 were characterized by intermediate values (2.4 and 3.6 h, respectively). The half-dissociation times of the complexes were similar for all examined LA (Table 1). This fact probably reflects comparatively low affinity of HAS for LA: the corresponding dissociation constants vary from 3 μM for bupivacaine to 25 μM for lidocaine [9].

Our experiments demonstrated significant differences in the kinetics of HAS complexation with various LA. These findings can be used as experimental substantiation for preparing HAS—LA mixtures in pharmaceutics.

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