Effect of Salicylates on the Toxicity of GABA-Lytics in Mice

A. I. Golovko, G. A. Sofronov, and T. V. Klyuntina

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Preliminary injection of aspirin and salicylic acid increases the toxicity of picrotoxin, but not of bicuculline. Aspirin inhibits binding of ^{3}H-diazepam and N-methyl-^{3}H-methylphenazepam with membranes from the brain of intact mice. In view of the fact that salicylates reduce the content and sorption capacity of serum albumin, this protein can be assumed to be involved in the detoxication of picrotoxin.

Key Words: *GABA-lytics; salicylates; toxicity; receptors; serum albumin*

GABA-lytics represent a class of chemical compounds inhibiting GABA-ergic neurotransmission [6,7]. There are some data on the involvement of blood and liver carboxyesterases [3] and microsomal monooxygenases [8] in the detoxication of these toxins. Albumin is known to be able to bind various xenobiotics [4]; however, the role of serum albumin in the neutralization of GABA-lytics remains unclear.

To elucidate this in the present study we evaluated the toxicity of the GABA-lytics picrotoxin (PT) and bicuculline (BC) against the background of preliminary injection of aspirin and salicylic acid, which reduce the sorption capacity and inhibit the synthesis of serum albumin [4,5,10].

MATERIALS AND METHODS

The experiments were performed on male white mice weighing 22-25 g. PT (Serva) was suspended in physiological saline using Tween-80. BC (Sigma) was dissolved in 0.1 N HCl and the pH of the solution was adjusted to 5-6 with 1 N NaOH [9]. Aspirin and salicylic acid (domestic pharmacopoeic preparations) were dissolved in 20% dimethyl sulfoxide and injected in a dose of 200 mg/kg 30 min to 48 hours prior to GABA-lytics. The substances were injected intraperitoneally: 0.2 ml/10 g body weight for GABA-lytics and 0.1 ml/10 g body weight for salicylates. For evaluation of the toxicity no less than 6 animals were used for each dose and no less than 5 doses were tested. LD_{50} was calculated using regression analysis by the method of least squares.

We studied the effect of aspirin and salicylic acid (10^{-9}-10^{-4} M) on specific binding of ^{3}H-diazepam and N-methyl-^{3}H-methylphenazepam (V. G. Khlopin Radium Institute, St. Petersburg, 2.3-2.4 TBq/mM), both in a concentration of 3 nM, as well as ^{3}H-quinuclidinyl benzylate (QNB, New

TABLE 1. Effect of Salicylates (200 mg/kg, Intraperitoneally) on Toxicity of GABA-Lytics for Male White Mice

Conditions	LD_{50}, mg/kg
PT, control	5.06±0.71
Aspirin 30 min before PT	2.50±0.38**
Aspirin 6 hours before PT	3.29±0.31*
Aspirin 24 hours before PT	3.44±0.29*
Aspirin 48 hours before PT	4.25±0.37
Salicylic acid 30 min before PT	2.68±0.54**
Salicylic acid 3 hours before PT	2.70±0.53**
BC, control	4.68±0.88
Aspirin 30 min before BC	4.21±0.43
Aspirin 6 hours before BC	5.01±0.49
Aspirin 24 hours before BC	4.36±0.92

Note. *$p<0.05$, **$p<0.01$ in comparison with the control.

Military Medical Academy, St. Petersburg

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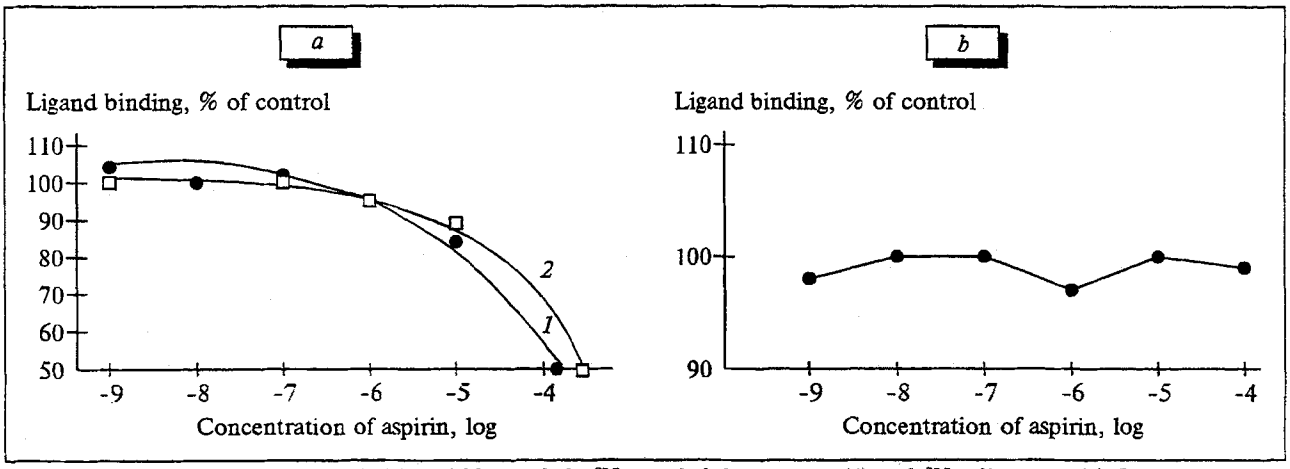

Fig. 1. Effect of aspirin (*a*) on binding of N–methyl–^{3}H–methylphenazepam (*1*) and ^{3}H–diazepam (*2*) (both in a concentration of 3 nM), and effect of salicylic acid (*b*) on binding of N–methyl–^{3}H–methylphenazepam with synaptic membranes from the brain of intact mice. Ligand binding in the control: N–methyl–^{3}H–methylphenazepam – 976 fmol/mg protein; ^{3}H–diazepam – 174 fmol/mg protein.

England Nuclear, 1.2 TBq/mM, 0.5 nM) and ^{3}H-methylscopolamine (Amersham, 3.5 TBq/mM, 0.5 nM) with synaptic membranes of the total brain of intact mice. Preparation of the membranes and the procedure of radioligand assay were described previously [1,2]. The reliability of the differences between compared parameters was evaluated using the Student *t* test.

RESULTS

Table 1 presents data on the toxicity of PT and BC in mice preinjected with aspirin and salicylic acid in a dose of 200 mg/kg. As is seen from the table, under these conditions the toxicity of PT (but not of BC) increased. For instance, 30 min, and 6 and 24 hours after injection of aspirin, LD_{50} for PT was decreased by 51%, 35%, and 32%, respectively. Preliminary injection of salicylic acid also increased the toxicity of PT: by 47% after 30 min and 3 hours.

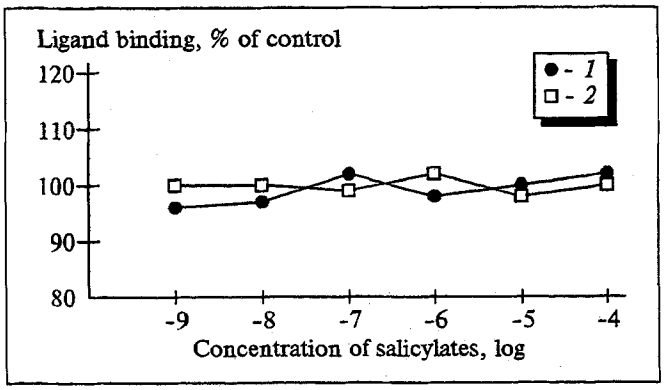

Fig. 2. Effect of aspirin on binding of ^{3}H–QNB (*1*) and ^{3}H–methylscopolamine (*2*) (both in a concentration of 1 nM) with synaptic membranes from the brain of intact mice. Ligand binding in the control: ^{3}H–QNB – 764 fmol/mg protein; ^{3}H–methylscopolamine – 905 fmol/mg protein.

Taking into account the possibility of using salicylates to act directly upon the GABA-benzodiazepine receptor complex, we assessed their effect on specific binding of ^{3}H-diazepam and N-methyl-^{3}H-methylphenazepam with synaptic membranes from the brain of intact mice (Fig. 1).

Aspirin inhibited binding of both ligands of the benzodiazepine receptors. The apparent IC_{50} was 2.04×10^{-4} and 1.24×10^{-4} M for ^{3}H-diazepam and ^{3}H-methylphenazepam, respectively (Fig 1, *a*). Salicylic acid had no effect on receptor binding of ^{3}H-methylphenazepam (Fig. 1, *b*).

The specific blockade of benzodiazepine receptors by aspirin is confirmed by the fact that binding of the ligands of muscarine receptors ^{3}H-QNB and ^{3}H-methylscopolamine under these conditions remained unchanged (Fig. 2).

Aspirin is known to lower the blood content of serum albumin in mammals [10]. Moreover, aspirin and its main metabolite salicylic acid bind to hydrophobic regions of the protein, thus reducing its sorption capacity [4,5]. The increased toxicity of PT against the background of preinjection of salicylates may imply the involvement of serum albumin in detoxication of this toxin. An effect of aspirin on the state of the GABA-benzodiazepine receptor complex cannot be ruled out either. This assumption is bolstered by the ability of salicylate to inhibit the binding of ^{3}H-diazepam and ^{3}H-methylphenazepam to synaptic membranes from the brain of intact mice. For salicylic acid the above mechanism of increased PT toxicity is less likely. The effects of salicylates on the microcirculation, the immunocompetent systems, and various biochemical processes should also be taken into account [5].

Thus, the increased toxicity of PT against the background of aspirin and salicylic acid reducing

the sorption capacity of serum albumin suggests that this protein plays a part in the detoxication of the GABA-lytic. For aspirin, however, another mechanism is also possible: alteration of the functional state of the GABA-benzodiazepine receptor complex.

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Substrate Supply to the Heart during Myocardial Infarction: Selectivity and Time Course of Utilization of Metabolites

F. N. Gil'miyarova, V. Yu. Golenishchev, and V. M. Radomskaya

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Myocardial infarction is experimentally modeled in rabbits. The blood content of metabolites in intact and experimental animals is measured at different times of infarction. An inverse utilization of the substrates by the myocardium is found: an enhanced utilization of glucose and reduced utilization of fatty acids. The emergence of metabolic acidosis and of an arrhythmogenic effect, and the aggravation of ischemia are substantiated by the accumulation of oxaloacetate, pyruvate, lactate, glycerophosphate, and dihydroxyacetone phosphate. During the repair period a tendency toward a normalization of substrate utilization by the myocardium is noted.

Key Words: *carbohydrate and lipid metabolism; myocardial infarction*

The contractile, self-regulating, and secretory functions of the myocardium are governed by intensive metabolic processes. Higher fatty acids have proved to be the primary substrate which supplies the myocardium with energy, endogenous water, and equivalents for the reactions of reductive synthesis [5]. At the same time, under certain conditions free fatty acids (FFA) are able to aggravate ischemia and exert an arrhythmogenic effect [7].

Being an intermediate in carbohydrate, lipid, and protein metabolism, oxaloacetate acts as an energetic precursor and assists (as a component of the malate-aspartate shuttle) the transport of cytoplasmic NADH into mitochondria. The acidic properties and polarity of the molecule are responsible for the electric balance of the medium, while two possible con-

Department of Biological and Clinical Chemistry, Samara State Medical University. (Presented by T. T. Berezov, Member of the Russian Academy of Medical Sciences)

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