Frequency-amplitude characteristics of the total biopotentials of the hippocamp ca3 zone under the influence of acetylsalicylic and salicylic acids

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Abstract. Neurotropic effects of the acetylsalicylic and salicylic acids at the perfusion of the rats’ hippocamp’s sections with the solutions of these acids in three concentrations $10^{-3}$ M, $10^{-5}$ M and $10^{-7}$ M are researched. It was found out that in all the cases extracellular total gigantic depolarizing biopotentials of the rats’ hippocamp CA3 zone are inhibited with the increase in the suppression intensity at the growth of the concentration of the researched chemical agents. In the range of 20-40 $\mu$V, 41-60 $\mu$V, 61-80 $\mu$V, 81-100 $\mu$V and 100+ $\mu$V most amplitude potentials were inhibited most intensely, at reaching the concentration of acid $10^{-3}$ M only potentials with the amplitude 20-40 $\mu$V were generated. Besides, the inhibiting effect of the salicylic acid excels the same of the acetylsalicylic acid. Comparing the results of this research with the previous ones, it has been found out that the effects of the researched acids are revealed unilaterally both in the nervous system of mollusks and rats.

1. Introduction

In the middle of 90-ies it was found out that aspirin could have an antidepressant effect, this immediately attracted the attention of the scientific community [1]. In further researches [2–4] convincing proofs of antidepressant and anxiolytic effect of this medicine and its derivatives were obtained. It is demonstrated that an antidepressant effect of aspirin is connected with its anti-inflammatory action [5]. But the main “stumbling block” is the fact that aspirin has a noticeable antidepressant effect at big doses or during long-term use in usual therapeutic doses [6]. In view of this a wide search for new promising compounds is being done; a special focus is on different modifications of the initial molecules [7]. Undoubtedly, any psychoactive action has either direct or indirect influence on the nervous system and its structural elements. But for an adequate and full assessment of the neurotropic effects of the mentioned acids’ derivatives it is necessary to have a clear and structured notion of these effects of the acetylsalicylic and salicylic acids per se.

It is also necessary to highlight that the researches of aspirin’s influence on the nerve cell, especially at the fundamental level, remain single.

2. Materials and methods

To prepare in vitro preparations of the hippocamps, experiencing sections, laboratory rates of Wistar line at the age of 14-16 months were used. After the decapitation the animal’s brain was extracted and then put into the cooled (4° C) solution of the artificial cerebrospinal liquid (aCSF) with the following composition (in $\mu$M): NaCl 126, KCl 3.5, CaCl2 2.0, MgCl2 1.3, NaHCO3 25, NaH2PO4 1.2, glucose
11 (pH 7.4), saturated with oxygen (95%) and carbonic acid (5%). Then the cerebellum was removed and the hippocamp horizontal sections with the thickness 400 μm were prepared on the microtome 7000 SMZ-2 Campden Instruments. The obtained sections were cut into two hemispheres and the ready preparations were put into the oxygenic (95% O₂/5% CO₂) solution aCSF, in which they were kept at the room temperature (20-22°C) for about an hour before the immediate usage in the experiment.

For the registration of the biopotentials the specimen was placed in a special dish with the continuous stream (2ml/min) of the oxygenic solution CSF. The immediate registration of the extracellular biopotentials was done with the help of a metallic (chlorine-silver) electrode connected with the amplifier Model 2400 A-MSysytem, the signal digitization was done by the analog-to-digital converter CED 1401 Micro3. The temperature of the washing solution was maintained at the level of 37°C by the thermocontroller TC-324C. The extracellular net activity was registered in CA3 hippocamp zone. In general the experiment methodically corresponded to generally accepted approaches [8], but we analyzed the most expressive (in essence gigantic depolarizing potentials), having divided them into five amplitude categories: 20-40 μV, 41-60 μV, 61-80 μV, 81-100 μV and 100+ μV.

The researched acetylsalicylic and salicylic acids were perfused in the concentrations of 10⁻³ M, 10⁻⁵ M and 10⁻⁷ M, immediately the perfusion with the acid solution lasted during 10 minutes, then the thirty-minute-washing followed. For each concentration of each acid 8 hemispheres of experiencing sections of the hippocamp were used. The record and analysis of the neurograms was done with the help software package Spike2. The statistic analysis was done by using the Tukey criterion.

3. The research results
In the current research at the extracellular lead the total biopotentials of the rats’ hippocamp CA3 zone were registered at the sections perfusion with the solutions of the acetylsalicylic and salicylic acids in three concentrations 10⁻³ M, 10⁻⁵ M and 10⁻⁷ M.

3.1. Let us start scrutinizing the obtained results with the description of the acetylsalicylic acid’s effects
In the concentration of 10⁻³ M the mentioned acid inhibited pronouncedly the generation of the biopotentials, causing considerable lowering of their average amplitude (Figure 1A) from 59±1.5 μV to 22.8±1.7 μV (p≤0.01) in 10 minutes from the beginning of the perfusion. After 30 minutes of washing the average amplitude of the biopotentials partially restored to 36.4±3.1 μV (p≤0.01), but still remained reliably less in comparison with the background index.

![Figure 1](image-url). The value of the average amplitude (A) and the frequency-amplitude distribution (B) of the total biopotentials of the hippocamp CA3 zone at the section perfusion with the acetylsalicylic acid solution in the concentration 10⁻³ M.

Note: ** - the differences of the index from the background level at p≤0.01, *** - the differences of the index from the background level at p≤0.001, •• – the differences of the index from its value after 30 minutes of washing at p≤0.01, asp10⁻³ 10 min – is the index value on the 10th minute of the section’s perfusion with the acetylsalicylic acid solution in the concentration 10⁻³ M, washing – is the value of the index after 30 minutes of washing.
While considering the frequency amplitude characteristics of the biopotentials it is seen that five types of the biopotentials with the amplitude 20-40 μV, 41-60 μV, 61-80 μV, 81-100 μV and more than 100 μV (Figure 1 B) are singled out on the background. The frequencies of this potentials are distributed as follows: 0.62±0.0081 Hz, 0.13±0.0057 Hz, 0.213333±0.012 Hz, 0.12±0.0051 Hz, 0.06±0.0025 Hz. After 10 minutes of the perfusion the activity of the section is substantially inhibited and only the biopotentials with the least amplitude 20-40 μV remained, and their frequency lowered to 0.028±0.003 Hz (p≤0.001).

In the concentration of the acetylsalicylic acid 10^{-5} M the inhibition of the section electric activity was observed, but the evidence of this inhibition was less in comparison with the concentration 10^{-3} M. Therefore, the lowering of the average amplitude (Figure 2 A) of the biopotentials from 59±1.5 μV to 26.2±1.2 μV (p≤0.01) after 10 minutes from the perfusion’s beginning was observed. After 30 minutes of the washing the average amplitude of the biopotentials partially restored to 38.2±1.3 μV (p≤0.01).

![Figure 2.](image1.png)

**Figure 2.** The value of the average amplitude (A) and the frequency-amplitude distribution (B) of the total biopotentials of the hippocamp CA3 zone at the section perfusion with the acetylsalicylic acid solution in the concentration 10^{-5} M.

Notes: asp10^{-5} 10 min – the value of the index on the 10th minute of the section perfusion with the acetylsalicylic acid solution in the concentration 10^{-5} M; the rest designations are the same as in Figure 1.

The frequency-amplitude distribution of the extracellular total potentials after 10 minutes from the perfusion beginning demonstrated the following: the biopotentials with the amplitude 20-40 μV and the frequency 0.49±0.03 (p≤0.01) Hz and the amplitude 41-60 μV and the frequency 0.02±0.002 Hz at p≤0.01 (Figure 2 B) continued generating. After 30 minutes of the washing the biopotentials with the amplitude more than 80 μV were generated very rarely.

The acetylsalicylic acid in the concentration 10^{-7} M kept the inhibiting effect on the bioelectrical activity of the section. The lowering of the average amplitude (Figure 3 A) of the biopotentials from 59±1.5 μV to 30.5±2.3 μV (p≤0.01) after 10 minutes from the perfusion’s beginning was observed. After 30 minutes of the washing the average amplitude of the biopotentials partially restored to 37.8±2.1 μV (p≤0.01).
Figure 3. The value of the average amplitude (A) and the frequency-amplitude distribution (B) of the total biopotentials of the hippocamp CA3 zone at the section perfusion with the acetylsalicylic acid solution in the concentration $10^{-7}$ M.

Notes: asp$10^{-7}$ 10 min – the value of the index on the 10th minute of the section perfusion with the acetylsalicylic acid solution in the concentration $10^{-7}$ M; * - the differences of the index from the background level at $p \leq 0.05$, • – the differences of the index from its value after 30 minutes of washing at $p \leq 0.05$, the rest designations are the same as in Figure 1.

Concerning the distribution of the potentials according to the amplitude ranges, used in the work, after 10 minutes from the beginning of the section’s perfusion with the acetylsalicylic acid solution in the concentration $10^{-7}$ M the biopotentials with the amplitude of 20-40 $\mu$V and the frequency $0.56 \pm 0.05$ Hz ($p \leq 0.05$), the amplitude 41-60 $\mu$V and the frequency $0.088 \pm 0.02$ Hz ($p \leq 0.05$) and the amplitude 61-80 $\mu$V and the frequency $0.027 \pm 0.007$ Hz ($p \leq 0.01$) continued generating. The total biopotentials with the amplitude more than 80 $\mu$V to the 10th minute of perfusion completely had disappeared.

After 30 minutes of the washing, the generation of the whole amplitude biopotentials’ spectrum restored, but most of its part was generated with a reliably less frequency in comparison with the background indices. As it is seen in Figure 3 B, after the washing the generation of the biopotentials of two amplitude ranges 20-40 $\mu$V and 41-60 $\mu$V according to the frequency indices corresponded to the background, but in the rest cases the frequency indices had reliably less values.

3.2. Neurotropic effects of the salicylic acid

Thus, in the concentration $10^{-3}$ M the salicylic acid caused a complete inhibition of the total biopotentials generation by the 10th minutes of the section perfusion (Figure 4 A). After 30 minutes of the washing the generation of the biopotentials resumed, and their average amplitude was $31.8 \pm 3.1$ $\mu$V ($p \leq 0.01$), being reliably lower than of the background level. While scrutinizing the frequency-amplitude characteristics of the biopotentials (Figure 4 B), it is also seen that by the 10th minute of the perfusion all the frequency indices are nullified. After 30 minutes of the washing the generation of the total biopotentials of only two amplitude ranges 20-40 $\mu$V with the frequency $0.17 \pm 0.02$ Hz and 41-60 $\mu$V with the frequency $0.038 \pm 0.002$ Hz resumed. Besides, the mentioned frequencies were noticeably lower than the background ones at the level of the value $p \leq 0.001$ and $p \leq 0.01$, correspondingly.
Figure 4. The value of the average amplitude (A) and the frequency-amplitude distribution (b) of the total biopotentials of the hippocamp CA3 zone at the section perfusion with the salicylic acid in the concentration $10^{-3}$ M.

Notes: SA$10^{-3}$ 10 min – the value of the index on the 10th minute of the section perfusion with the salicylic acid in the concentration $10^{-3}$ M; the rest designations are the same as in Figure 1.

At the section perfusion with the salicylic acid in the concentration $10^{-5}$ M, as expected, the inhibiting effect remained, but it became weaker. Thus, in 5 minutes of the perfusion the average amplitude of the total potentials remained above zero and was $20.8\pm1.8 \, \mu V$ at $p \leq 0.001$ in comparison with the background value. After the washing the average amplitude of the total potentials remained considerably lower in comparison with the background level and was $27.5\pm3.3 \, \mu V$ at $p \leq 0.01$.

The frequency-amplitude distribution of the total biopotentials of the hippocamp CA3 zone at the section perfusion with the salicylic acid solution in the concentration $10^{-5}$ M also demonstrated considerable inhibiting effect of the used chemical agent. By the 5th minute of the perfusion the potentials of all the amplitude ranges disappeared, except the least – 20-40 $\mu V$, which continued generating with the frequency $0.11\pm0.013$ Hz at a considerably lower level ($p \leq 0.01$) in comparison with the background (Figure 5A). After the washing the total potentials of two amplitude ranges manifested themselves: 20-40 $\mu V$ with the frequency $0.17\pm0.024$ Hz and 41-60 $\mu V$ with the frequency $0.038\pm0.0042$ Hz. Besides, the mentioned frequencies remained noticeably lower than of the background at the value level $p \leq 0.01$.

Figure 5. The value of the average amplitude (A) and the frequency-amplitude distribution (B) of the total biopotentials of the hippocamp CA3 zone at the section perfusion with the salicylic acid solution in the concentration $10^{-5}$ M.

Notes: SA$10^{-5}$ 10 min – the value of the index on the 10th minute of the section perfusion with the salicylic acid solution in the concentration $10^{-5}$ M; the rest designations are the same as in Figure 3.

At the section perfusion with the salicylic acid solution in the concentration $10^{-7}$ M the inhibiting effect was still pronounced enough (Figure 6 A). Thus, after 10 minutes of the perfusion the average amplitude of the total potentials was $25.8\pm1.5 \, \mu V$ at $p \leq 0.001$ in comparison with the background value. After the washing the average amplitude of the total potentials was $35.7\pm1.7 \, \mu V$ at $p \leq 0.01$. 
Figure 6. The value of the average amplitude (A) and the frequency-amplitude distribution (B) of the total biopotentials of the hippocamp CA3 zone at the section perfusion with the salicylic acid solution in the concentration $10^{-7}$ M.

Notes: SA $10^{-7}$ 10 min – the value of the index on the 10th minute of the section perfusion with the salicylic acid solution in the concentration $10^{-7}$ M; the rest designations are the same as in Figure 3.

The frequency-amplitude (Figure 6 B) distribution of the total biopotentials of the hippocamp CA3 zone at the section perfusion with the salicylic acid solution in the concentration $10^{-7}$ M was as follows: after 10 minutes of the perfusion with a significant ($p \leq 0.01$) less frequency the potentials of two ranges 20-40 μV (0.36±0.032 Hz) and 41-60 μV (0.049±0.003 Hz) were generated; after the washing the frequency of these potentials’ generation returned to the background level and the potentials 61-80 μV with the frequency 0.061±0.018 Hz at $p \leq 0.01$ appeared.

4. Discussion

The results of this research convincingly demonstrate the inhibiting neurotropic effect of the acetylsalicylic and salicylic acids. It is perfectly predictable that the inhibition intensifies in the range of the used concentrations from $10^{-7}$ M to $10^{-5}$ M. Comparing the effects of these two acids, we can point with confidence at the inhibiting effect of the salicylic acid which is more pronounced in comparison with the acetylsalicylic acid. This conclusion coincides with the scientific literature data, which show that the salicylic acid according to many inhibiting effects excels the effect of the acetylsalicylic acid [9]. Also, the results of the neurotropic influence of the researched acids coincide with the ones, obtained earlier on the nervous system of the invertebrates [10, 11].

It is necessary to mention that under the influence of the acids first the total biopotentials of the high-amplitude spectrum from 60 μV and more are inhibited (disappear). In the case of the extracellular lead the amplitude of the potentials is determined by the amount of relatively synchronously activating neurons, so under the influence of the acids their quantity per unit time considerably decreases, right up to null under the influence of the salicylic acid in the concentration $10^{-3}$ M.

One more interesting aspect of the work is the following fact: the used acids influence the cycle of the arachidic acid, blocking cyclooxygenase (COG). Besides, in high concentrations (about $10^{-3}$ M) the researched salicylates blockade two types of the ferments COG-1 and COG-2; in lower concentrations – only COG-2 [12]. That is why the observed effects can be conditioned by the different intensity of the COG blockade. We also consider that quite a significant role in the neurotropic effects of the acetylsalicylic and salicylic acids are played by the ATP synthesis inhibition [11]. Therefore, the obtained results can be interpreted as the effect of differently manifested COG blockade and the blockade of ATP synthesis. Such an assumption is proved by the scientific literature sources, in which the authors point out the significance of the arachidic acid system in the functioning of the neural nets and the highest functions connected with them [13 – 15].

From the position of the ionic mechanisms, we consider that the most probable is the inhibition of the input sodium current and the processes of synaptic transmission by the used acids. The identification of these biophysical mechanisms is the priority task of our further research.
5. Conclusions
The acetylsalicylic and salicylic acids have a unidirectional inhibiting effect on the net of neuron bioelectrogenesis of the rat’s hippocamp, which is mostly pronounced in the salicylic acid. The orientation of the researched acids’ effects corresponds to the results, obtained on the neurons of the mollusks; this indicates the universality of these solutions’ influence.

6. References
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