Change of electromyographic parameters in rats after injection of acetyl salicylic acid and its complex compounds with metals

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Abstract. The paper studies the identification of the acetylsalicylic acid (ASA) effects and salicylates of cobalt and nickel at different doses from 5 mg / kg to 20 mg / kg (increase step by step 5 mg/kg) by patterns muscle activity of needle electromyography (EMG) in rats. The analysis of the parameters needle electromyogram showed that after the injection of ASA at different doses most significant increase of the maximum and total amplitudes, average frequency, and minimum duration of PMU is observed. It is also noted that the growth of spectral parameters indicates an increase in the degree of impulses synchronization of different motor units. When rats were injected with ACNi²⁺, EMG, indices changed in the similar way, but less significantly. The injection of ACCo²⁺ at a dose of 5 g / kg in animals caused a significant increase of the main indicators of the turn-amplitude analysis in comparison with the control group values. The injection of this compound at doses of 10 and 20 mg/kg caused a significant decrease in the studied EMG parameters; this indicates the decrease in the motor neurons activity.

1. Introduction
In contemporary scientific literature, there is an increased interest to the study of neurophysiological mechanisms of motor activity regulation [1-3]. This is important for understanding the adaptation of the humans and animals to various conditions of its vital activity. The solution of this multidisciplinary problem is of great interest for physiology, clinical and rehabilitation medicine.

It is known that one of the basic electrophysiological methods for diagnosing disorders of neuromuscular transmission is the method of electromyography (EMG), including the needle myography. The needle EMG is an objective method for getting data about interaction and levels of the working muscles activity, the functioning of nervous structures [3-7]. There is a significant number of methods for processing an EMG signal (visual assessment method, turn-amplitude, spectral using the method of fast Fourier transforms, recute quantitative, fractal analyzes, etc.). It makes possible to study the state of the neuromuscular system in details, identify new patterns of movement with a redistribution of different muscle groups’ involvement [5, 8-10].

In the world neurological practice the diagnostics issues and treatment of diseases, accompanied by impaired neuromuscular transmission with degenerative-dystrophic lesions of motor neurons, myopathies of various origins, etc., which lead to profound disability and even death, remain open [11, 12]. This predetermines the need for search for new effective drugs, as well as to study the mechanism of their action. Such means could be new chemical compounds of acetylsalicylic acid (ASA) with
divalent metals, which, along with an increase in the effectiveness of ASA, can significantly reduce the side effects inherent in the precursor molecule \([13-16]\).

Our previous studies have shown that metal salicylates containing complexing agents \(\text{Co}^{2+}\) and \(\text{Ni}^{2+}\) have not only more pronounced, but also qualitatively new, in comparison with ASA effects. In this case, the severity of the developing effect depends not only on the bimetal included in the complex compound with ASA, but also on the effective dose of the tested compounds \([18-19]\). At the same time, in the modern scientific literature there are no data on the effect of these compounds on the state of the neuromuscular system.

In this regard, the aim of this study was to identify the effects of ASA and salicylates \(\text{ACC}_2^{2+}\) and \(\text{ACNi}_2^{2+}\) on the parameters of muscle activity in rats at different doses of 5 mg / kg, 10 mg / kg and 20 mg / kg.

### 2. Materials and methods

Experimental studies were carried out on the basis of the Center for Shared Use of Scientific Equipment "Experimental Physiology and Biophysics" at V.I. Vernadsky Crimean Federal University, the Department of Human and Animal Physiology and Biophysics.

100 laboratory sexually mature male rats of the Wistar line, weighing from 190 to 240 grams, were chosen for the experiments ("Federal State Unitary Enterprise" Nursery of laboratory animals "Rappolovo"). The animals were kept in standardized vivarium conditions at temperatures from 18 to 22\(^{\circ}\)C, on a Rehofix MK 2000 bedding (based on corn cobs) with natural illumination of a 12-hour light-dark cycle. There was an access to water in accordance with State Standard 33215-2014 “Guidelines for the maintenance and care of laboratory animals. Rules for the equipment of premises and organizational procedures” and to full-fledged granulated feed State Standard R-50258-92.

In the course of the experiment a study of the biological effectiveness of three compounds - ASA, \(\text{ASC}_2^{2+}\) and \(\text{ASN}_2^{2+}\) (purity not less than 98.0%; synthesized at the Department of General and Inorganic Chemistry of the V. I. Vernadsky Crimean Federal University) was carried out.

All the animals were divided into 10 equal groups of 10 animals in each as follows:

- **Group 1** - control (C) - this group received intraperitoneal injections of physiological solution (NaCl, 0.9%) with a volume of 0.2 ml;
- **Groups 2-4** - ASA - the animals of this group received intraperitoneal injections of ASA at different doses (in a volume of 0.2 ml);
- **Groups 5-7** - \(\text{ACNi}_2^{2+}\) - the groups of animals that received of nickel salicylate at different doses (in the volume of 0.2 ml);
- **The **\(\text{ACC}_2^{2+}\) groups 8-10** - these groups were injected with cobalt salicylate at different doses (in a volume of 0.2 ml).

20 minutes after the injection of these compounds in the anesthetized animals (inhalation of isoflurane; Baxter Pharmaceutical Solutions, LLC, USA), EMG was recorded in the "Interference EMG" and "Registration potentials of motor unit (PMU)" test modes for 3 minutes each on a computer 4-channel electroneuromyograph "Neuro-MVP.NETomega" ("NeuroSoft", Russia, Ivanovo). The installation of the electrodes was carried out as follows: a ground electrode from the outer side of the animal's thigh surface was connected subcutaneously; a recording concentric electrode was connected from the inside intramuscularly into the thigh area in m. gracilis at a depth of 0.7 - 0.9 mm \([19]\).

For the first time the EMG curve was visually analyzed. After the registration of EMG surface, there were applied quantitative methods, including computerized, EMG processing methods, which include the following indicators of turn-amplitude analysis of EMG: maximum, average, total amplitude (μV) and average signal frequency according to turn-amplitude analysis data (1 / s).

The registration of PMU was carried out with minimal voluntary muscle contraction. There are main indicators of PMU: the values of duration, amplitude and shape of PMU (reflect the size and number of motor units), the relative position of muscle fibers and the density of their distribution in each specific MU \([20]\).
The spectral analysis parameters of the EMG (mean frequency (Hz), median frequency (Hz) and spectrum power (1400 Hz, 1/million)) were analyzed by determining the frequency-energy characteristics of the bioelectric muscle activity as a result of the fast Fourier transform of bioelectric activity fragment with numerical and graphic representation of the final result [21, 22].

The calculations, statistical processing and graphic presentation of the data obtained in the work were carried out using Microsoft Excel and StatSoft / STATISTICA 8. The significance of differences in the mean values of EMG indices obtained in the animals of the control and experimental groups was assessed using ANOVA analysis using the F-test. Fisher (Repeated measures ANOVA or GLM-4).

3. Results and discussion

The results of the needle EMG turn-amplitude analysis of the first control group animals showed the following values: maximum, average and total EMG amplitudes - 2084.00 ± 540.21 μV, 519.33 ± 96.67 μV and 147.39 ± 39.39 mV / s, respectively, the average frequency of EMG turns is 280.78 ± 36.79 1 / s. The minimum and maximum PMU amplitudes were registered - 480.00 ± 364.67 μV and 1767.00 ± 708.45 μV; the minimum and maximum PMU durations were 9.76 ± 2.96 ms and 19.91 ± 4.46 ms, respectively. Spectral analysis indices had the following values: mean frequency - 123.74 ± 28.44 Hz, median frequency - 94.07 ± 11.97 Hz, spectrum power - 128.10 ± 72.30 1 / mln. (Figure 1).

The obtained EMG results were within the normative values and are consistent with the scientific literature data.

Visual assessment of the EMG curve of the first group at rest corresponded to type I normal saturated EMG of tonic rest according to the classification proposed by Yu.S. Yusevich [6].
3.1. Changes in EMG indices in the rats after injection of ASA at different doses

When the rats were injected with ASA at a dose of 5 mg / kg, a statistically significant increase in the maximum EMG amplitude by 39.03% (p≤0.01) was registered relative to the data recorded in the animals of the control group. Other indicators of turn-amplitude analysis (mean, total amplitudes and mean frequency) also tended to increase in comparison with the corresponding indicators in the control (Figure 1, 2).

The injection ASA at a dose of 10 mg / kg in the animals, there was a statistically significant increase in the indicators of the maximum and total EMG amplitudes by 37.40% (p≤0.01) and 42.37% (p≤0.01), respectively, relative to the values of the indicators, registered in the animals of the control group. There was also a significant increase in the average frequency of EMG turns by 35.81% (p≤0.01) relative to the corresponding values in the control (see Figure 1, 2).

![Figure 2. Changes in the average frequency (1 / s) of electromyogram in the rats after injection of acetylsalicylic acid (ASA) and salicylates (AS) Ni²⁺ and Co²⁺ at the different doses (in comparative with the control group). * - reliability of differences by the ANOVA method.](image)

When the rats of group 4 were injected with ASA at a dose of 20 mg / kg, all the indicators of turn-amplitude analysis changed at the level of a trend relative to the corresponding values in the control animals.

Analysis of PMU indicators needle EMG in the rats after injection of ASA at a dose of 5 mg / kg revealed an insignificant increase in amplitudes (minimum - by 6.63% (p ≥ 0.05), maximum - by 4.02% (p ≥ 0.05)). There was statistically significant increase in duration (minimum - by 35.73% (p≤0.01) and average - by 19.87% (p≤0.05)) muscle PMU relative to the values of the corresponding indicators in animals in the control group (Figure 3).

With the increase in the dose of ASA to 10 mg / kg, the PMU EMG indices changed insignificantly, with the exception of the maximum amplitude of the PMU of the muscle, which increased by 28.21% (p≤0.01) relative to the values in the animals in the control group.

The analysis of the main indicators of spectral indicates in the animals, that were injected with ASA at a dose of 5 mg / kg, revealed a statistically significant increase in the average frequency (by 26.63%, p≤0.01), as well as the spectrum power (by 56.45%; p ≤0.01) EMG relative to control values (Figure 4, 5). Moreover, the values of these indicators were significantly higher than the corresponding data recorded in the animals that were injected with ASA 10 and 20 mg / kg.

Thus, the most pronounced changes in EMG parameters were observed in the animals that received ASA at doses of 5 and 10 mg / kg.
Figure 3. Indicators of the amplitude (minimum, maximum and average) (A) and duration (minimum, maximum and average) (B) PMU EMG in the rats with the injection of acetylsalicylic acid (ASA) and salicylates (AS) Ni$^{2+}$ and Co$^{2+}$ at the different doses (in comparative with the control group).

* - reliability of differences by the ANOVA method.

3.2. Changes in EMG indices in the rats after administration of ACNi$^{2+}$ at different doses

The comparison indicators in the animals exposed to ASA injection at a dose of 5 mg / kg, with the injection of ACNi$^{2+}$ at the same dose, a statistically significant decrease in the maximum EMG amplitude by 25.65% ($p \leq 0.01$) was recorded (see Figure 1).

The injection of ACNi$^{2+}$ into the animals at a dose of 10 mg / kg caused a significant decrease in the indicators of the maximum (by 30.09%; $p \leq 0.02$) and total (by 27.32%; $p \leq 0.05$) EMG amplitudes relative to the values of the corresponding indicators in animals, which were injected with ASA at the same dose. The increase in the ACNi$^{2+}$ dose to 20 mg / kg led to statistically insignificant changes in EMG indices in comparison with those recorded in the group of animals that received ASA 20 mg / kg (see Figure 2).

Figure 4. Indicators of spectral analysis of EMG (mean frequency, median frequency of the spectrogram and spectrum power) in the rats after injection of acetylsalicylic acid (ASA) and salicylates (AS) Ni$^{2+}$ and Co$^{2+}$ at doses of 5, 10 and 20 mg / kg (in% relative to the control values).

* - reliability of differences in comparison with the data obtained in the animals of the control group using analysis of variance ANOVA.
The analysis of PMU indices in the animals that were injected with ACNi$^{2+}$ at doses of 5 mg / kg and 20 mg / kg showed a tendency (p ≥ 0.05) to decrease in the minimum amplitude and minimum duration and increase in the maximum amplitude and maximum duration of PMU in comparison with the corresponding indicators in the control animals. The injection of ACNi$^{2+}$ into the animals at a dose of 10 mg / kg caused a statistically significant increase in the minimum amplitude (by 36.46%; p≤0.02) and minimum duration (by 28.93%; p≤0.05). It also caused a decrease in the maximum amplitude of the PMU (by 23.43%; p≤0.05) in comparison with the values of those indicators in the animals in the control (Figure 3).

The spectral analysis of needle EMG detected a significant increase in the EMG spectrum power readings with the injection of ACNi$^{2+}$ at a dose of 5 mg / kg (by 26.42%, p≤0.05). With the injection of this compound at a dose of 10 mg / kg, on the contrary, a decrease in this indicator (by 57.49%, p≤0.01) was detected in comparison with those in the animals of the control group (Figure 4, 6).

Thus, the injection of ACNi$^{2+}$ at different doses did not cause significant changes in the turn-amplitude analysis of EMG, the values of which approximated those in the animals of the control group. The most significant changes in spectral analysis indicators, and also PMU in comparison with the values of the corresponding indicators in the control, were noted when this chemical compound was injected at a dose of 10 mg / kg.

3.3. Changes in EMG indices in the rats after administration of ACCo$^{2+}$ at different doses

After the injection of ACCo$^{2+}$ at a dose of 5 mg / kg in the animals, there was a statistically significant increase in indicators of maximum (by 34.59%; p≤0.02), average (by 17.84%; p≤0.03) and total (by 19.95%; p≤0.01) EMG amplitudes relative to the corresponding values in the animals of the control group. They were approximate to those recorded ones in the animals that were injected with ASA at a dose of 10 mg / kg (see Figure 2).
A similar data were registered in the analysis of motor units indicators: in this group of animals, there was a tendency to an increase in both the amplitude and duration of the PMU in comparison with the indicators in the animals of the control group.

The injection of ACCo2⁺ at doses of 10 and 20 mg / kg caused a significant decrease in the parameters of the maximum, average, total amplitudes and average frequency of EMG relative to the corresponding values in the animals of the control group. The most significant changes were noted with the injection of ACCo2⁺ at a dose of 20 mg / kg. Thus, the indicator of the maximum amplitude decreased by 34.54% (p≤0.01); the average amplitude - by 55.37% (p≤0.01), the total amplitude - by 28.91% (p≤0.001), and average turn frequency - by 19.44% (p≤0.01) relative to the values in control (see Figure 2). The indicators of the minimum and maximum PMU in the rats of these groups also significantly decreased on average by 21.95% (p≤0.05) and 24.55% (p≤0.05), respectively, in comparison with the values recorded in the animals in the control group.

Thus, the administration of ACCo2⁺ into the animals at a dose of 5 mg / kg caused a statistically significant increase in the main electromyogram indicators, relative to the control group. The injection of this compound at doses of 10 and 20 mg / kg, on the contrary, led to a statistically significant decrease in the studied EMG parameters, which indicates the decrease in the activity of motor neurons.

Based on the discovered changes in the amplitude-frequency, spectral and parameters of the PMU of the needle EMG, it is possible to judge about the change in the functional state of the neuromuscular system of rats when the test compounds are injected at different doses. As the study results have shown, the most significant changes are characteristic of turn-amplitude indicators, spectral analyzes and indicators of the PMU of needle EMG (an increase in the maximum and total amplitudes, average frequency, minimum duration of PDE, spectral indicators), were recorded with the injection of ASA at a dose of 5 mg / kg.

According to most researchers, there is a close relationship between the indicators of turn-amplitude analysis of EMG and the strength indicators of muscles [9, 23].

The increase in the amplitude-frequency parameters of EMG upon injection of the tested compounds is explained by an increase in the frequency of impulses of MU at the level of the spinal cord and indicates an increase in the degree of synchronization of different MU impulses. In particular, it has been shown that the change in the indices of the total amplitude of EMG characterizes the strength abilities of the muscle and makes it possible to judge the average total activity of the correspondingly activated MU of the muscle [6]. The changes in this indicator directly depend on the number of recruited MUs and the degree of their synchronization, with an increase in muscle power indicators, the frequency of impulses of motor neurons also increases, and these MUs are recruited.

The increase in the amplitude and duration of PMU recorded after administration of ASA at doses of 5 and 10 mg / kg can indicate that the presynaptic and / or potential of the end plate was facilitated and that the dispersion of AP between fibers was reduced [24].

A direct relationship was also shown between the average frequency of the spectrum and the rate of conduction of excitation along the muscle fiber. This fact is used to detect muscle fatigue and to roughly determine the type of active MUs [25]. A rise in the spectral power of EMG also indicates an increase in the frequency of individual MUs impulses [26] and indicates the development of new adaptive reactions at the level of the neuromuscular system [9].

Other effects were observed with the injection of the tested metal salicylates. Thus, the injection of ACNi³⁺ into rats, the pattern of EMG changes was not so significant as with the injection of only ASA. At a dose of 10 mg / kg this chemical compound caused a significant increase in the amplitude and duration of PMU EMG relative to the values in the control. At a dose of ACNi³⁺ 5 mg / kg it was an increase in the indices of the EMG spectrum power, and with the injection of 20 mg / kg, on the contrary, there was a decrease in this indicator in comparison with those in the animals of the control group. These changes also indicate an increase in the MU impulse frequency, the degree of synchronization of impulses of different MUs at a dose of 5 mg / kg, and a higher dose of 20 mg / kg leads to a slowdown in the MU impulse frequency.
The injection of ACCo$^{2+}$ at a dose of 5 mg / kg in the animals caused a significant increase in the main indicators of turn-amplitude analysis relative to the corresponding data in animals of the control group. At the same time, these data approximated the values of the animals, which were injected with ASA 10 mg / kg.

The injection of this compound at doses of 10 and 20 mg / kg caused a significant decrease in the amplitude-frequency parameters, as well as the parameters of the MU; this indicates a decrease in the activity of MUs, the predominant activation of low-threshold MUs. A low-amplitude saturated, partially taut or stockade EMG” [27] is registered with this inhibition of motor neurons, resting activity and synergistic muscle activation decrease. That was observed when the compound was administered at a dose of 20 mg / kg. The data obtained may indicate damage to the roots, plexuses of peripheral nerves and anterior horns of the spinal cord [28].

Thus, the results of this study detected a multidirectional dose-dependent effects of ASA and its derivatives (ACNi$^{2+}$ and ACCo$^{2+}$) on the functional state of the experimental animals’ neuromuscular system.

4. Conclusions
1. Changes in indicators of turn-amplitude, spectral analysis and potentials of motor units of electromyogram were detected when the rats were injected with acetylsalicylic acid and salicylates of cobalt and nickel at doses of 5, 10 and 20 mg / kg.
2. The at doses of 5 and 10 mg / kg of the acetylsalicylic acid lead to the most significant increase in the maximum and total amplitudes, average frequency, minimum duration of motor unit potentials and spectral parameters was recorded. This indicates an increase in the degree of impulses synchronization of different nervous system motor units in the experimental animals muscular system.
3. The dose of 10 mg / kg ACNi$^{2+}$ leads to significant increase the main electromyogram indicators in comparison with control group. The dose of 20 mg / kg promotes to the decrease in the values of these indicators in comparison with the animals of the control group.
4. The injection of ACCo$^{2+}$ into the animals at a dose of 5 mg / kg caused a significant increase in the main indicators of turn-amplitude analysis relative to the corresponding data in the animals of the control group. An increase in the dosage to 10 and 20 mg / kg caused a significant decrease in the studied EMG parameters; this indicates a decrease in the activity of motoneurons of the experimental animals’ neuromuscular system.

5. References
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