Metal salicylates Co$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Li$^{+}$ and Mg$^{2+}$: properties and effect on pain sensitivity

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Abstract. The paper presents the results of evaluating the effect of salicylates Co$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Li$^{+}$ and Mg$^{2+}$ at doses of 5, 10 and 20 mg/kg on the pain sensitivity of male rats. Experiments were carried out on 119 male Wistar laboratory rats in test models of acute thermal pain (“tail-flick” and “hot plate”). It has been shown that acetylsalicylic acid (ASA) reduces the pain sensitivity with the participation of spinal and supraspinal mechanisms of regulation. The introduction of complexing metals (Co$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Li$^{+}$ and Mg$^{2+}$) mainly leads to a decrease in the analgesic effect of ASA.

1. Introduction
Acetylsalicylic acid (ASA) has been used for many years not only as a universal analgesic, antipyretic and anti-inflammatory agent [1], but also as a unique source for the production of a wide coordination compounds variety, allowing the synthesis of new derivatives with complexing metals and various biologically active molecules and ligands [2]. The new compounds based on ASA obtained in this way are devoid of its negative side effects and are successfully used as antitumor, anti-inflammatory and antimicrobial substances [3, 4].

Positive results of studies ASA derivatives biological effectiveness suggest that complexation based on divalent metals and salicylates is promising [4], since such derivatives with bimetals (Fe, Zn, Co, Cu, etc.) have fewer side effects and more pronounced effects than standard aspirin.

In this regard, the aim of this study was to identify the analgesic effects of salicylates (AS) Co$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Li$^{+}$ and Mg$^{2+}$ on the thresholds of pain sensitivity (PS) in rats.

2. Material and methods

2.1. Animals
The experimental part of the work was performed in the Center for Collective Use of Scientific Equipment “Experimental Physiology and Biophysics” of the Department of Human and Animal Physiology and Biophysics of the Taurida Academy (structural division of the V. I. Vernadsky Crimean Federal University). The animals participating in the experiment were kept in standard vivarium conditions at a temperature of 18-22 °C on the “Rehofix MK 2000” litter (based on corn cobs) with a natural 12-hour light-dark cycle, free access to water (State Standard 33215-2014...
“Guidelines for the maintenance and care of laboratory animals. Rules for the equipment of premises and the organization of procedures”) and full-fledged granulated feed State Standard R-50258-92. The study was conducted in accordance with State Standard R-53434-2009 “Principles of Good Laboratory Practice” and the rules set out in Directive 2010/63/EU of the European Parliament and of the Council of 22.09.2010 on the protection of animals used for scientific purposes.

2.2. Design of research
The experiments were performed on male Wistar laboratory rats weighing 200-250 g. They were divided into 22 groups of 7 individuals each.

- **Group 1** – control (K) – male rats received intraperitoneal (i. p.) injections of 0.2 ml of saline solution (0.9 % NaCl) and were in standard vivarium conditions.
- **Groups 2-4** – male rats received i. p. injections of 0.2 ml ASA in doses of 5, 10 and 20 mg/kg;
- **Groups 5-7** – male rats received i. p. injections of 0.2 ml cobalt salicylate (ASCo2+) in doses of 5, 10 and 20 mg/kg;
- **Groups 8-10** – male rats received i. p. injections of 0.2 ml nickel salicylate (ASNi2+) in doses of 5, 10 and 20 mg/kg;
- **Groups 11-13** – male rats received i. p. injections of 0.2 ml zinc salicylate (ASZn2+) in doses of 5, 10 and 20 mg/kg;
- **Groups 14-16** – male rats received i. p. injections of 0.2 ml manganese salicylate (ASMn2+) in doses of 5, 10 and 20 mg/kg;
- **Groups 17-19** – male rats received i. p. injections of 0.2 ml magnesium salicylate (ASMg2+) in doses of 5, 10 and 20 mg/kg;
- **Groups 20-22** – male rats received i. p. injections of 0.2 ml lithium salicylate (ASLi+) in doses of 5, 10 and 20 mg/kg.

The studied substances were synthesized under the guidance of Professor A. N. Gusev at the Department of General and Inorganic Chemistry (Faculty of Biology and Chemistry) of Taurida Academy (structural division), V. I. Vernadsky Crimean Federal University. The chemical purity of tested compounds was not less than 98.0 %.

Testing of PS thresholds in rats was performed 20 minutes after injection in the “tail-flick” and “hot plate” models of acute pain stress. Before the “tail-flick” test, animals were placed in special retainers for rats (AE1001-R0, Open Science, Russia).

In the “tail-flick” test evaluated the perceptual component of pain. The main indicator of this test was the tail-flick latency (TFL) in response to light-thermal irritation. TFL was determined by the value of the time (s) of the tail withdrawal reaction. TFL was measured on the device LE7106 Tail-flick Meter (Pan Lab Harvard Apparatus, Spain). On the tail of each rat, sitting in the fixator, three presentations of a thermal stimulus were performed, followed by the calculation of the average value of TFL in seconds for each animal. This test is based on a spinal flexor reflex that occurs in response to a local force on the tail by high temperature, and allows us to judge the PS of animals mainly at the spinal level [5-8].

In the “hot plate” test on experimental apparatus Cold and hot plate CHP (Bioseb, France), the hot-plate test latency (HPTL) of the animal was recorded, which was determined by the value of the time (s) of the reaction manifestation of withdrawal and licking of the limbs and (or) vocalization from the heated surface. The test allows to judge the PS of animals with the participation of supraspinal mechanisms [8-11].

2.3. Statistical methods and analysis of biological action with metals
Calculations, statistical processing and graphic design of the data obtained in the work were carried out using the Microsoft Excel program and the Graph Pad Prism 7.0 software package. The reliability of statistical differences between the control and experimental groups with different doses of ASA and metal salicylates was determined by using a one-way analysis of variance (ANOVA) with a posteriori Tukey test and Dunn’s test of multiple comparisons.
For analysis of AS biological action with metals effectiveness in comparison with the ASA, the efficiency coefficient (EC) was calculated using the formula:

$$EC = (AS_{met} - ASA)/ASA,$$

where $AS_{met}$ – the value indicators of PS thresholds registered in animals with the introduction of AS metals $Co^{2+}, Zn^{2+}, Ni^{2+}, Mn^{2+}, Li^+$ and $Mg^{2+}$; $ASA$ – indicators of PS thresholds registered in animals with the introduction of ASA.

The EC approaches zero if the efficiency of the studied compound ($AS_{met}$) in relation to the studied indicator corresponds to that for the ASA.

3. Results and discussion

The results of the study showed that the administration of ASA at doses of 5, 10 and 20 mg/kg significantly reduces PS in rats (Table 1). This is evidenced by a significant increase in TFL in the “tail-flick” test by 58.7 % (p<0.05), 80.4 % (p<0.05) and 114.4 % (p<0.05), respectively, and HPTL in the “hot plate” test by 61.1 % (p<0.05) at a dose of 10 mg/kg and 78.8 % (p<0.05) at a dose of 20 mg/kg relative to these parameters in the control group of rats.

Table 1. Changes in pain sensitivity thresholds in rats under the influence of salicylates $Co^{2+}, Zn^{2+}, Ni^{2+}, Mn^{2+}, Mg^{2+}$ and $Li^+$ in doses 5, 10 and 20 mg/kg.

| Substance | Dose (Group №) | TFL, s (M±m) | HPTL, s (M±m) |
|-----------|----------------|--------------|---------------|
| 0.9 % NaCl| control (1)    | 4.48±0.44    | 5.47±0.45     |
| ASA       | 5 mg/kg (2)    | 7.11±0.52a   | 7.40±0.50     |
| ASA       | 10 mg/kg (3)   | 8.08±0.45c   | 8.81±0.76b    |
| ASA       | 20 mg/kg (4)   | 9.61±0.92c   | 9.79±1.14c    |
| ASCo$^{2+}$| 5 mg/kg (5)    | 4.86±0.40d   | 4.74±0.52     |
| ASCo$^{2+}$| 10 mg/kg (6)   | 5.98±0.59e   | 4.64±0.25     |
| ASCo$^{2+}$| 20 mg/kg (7)   | 9.30±0.73c   | 9.94±0.61c,i  |
| ASNi$^{2+}$| 5 mg/kg (8)    | 6.06±0.57a   | 4.54±0.29     |
| ASNi$^{2+}$| 10 mg/kg (9)   | 4.94±0.20f   | 8.87±0.52c    |
| ASNi$^{2+}$| 20 mg/kg (10)  | 6.05±0.28a,i | 9.09±0.51c    |
| ASZn$^{2+}$| 5 mg/kg (11)   | 6.10±0.48    | 5.84±0.71     |
| ASZn$^{2+}$| 10 mg/kg (12)  | 6.53±0.47a,c | 7.34±0.98     |
| ASZn$^{2+}$| 20 mg/kg (13)  | 5.55±0.77h   | 7.07±0.64b    |
| ASMn$^{2+}$| 5 mg/kg (14)   | 6.34±0.45a   | 6.06±0.23     |
| ASMn$^{2+}$| 10 mg/kg (15)  | 9.96±0.69e   | 6.56±0.46c    |
| ASMn$^{2+}$| 20 mg/kg (16)  | 7.95±0.57c   | 8.08±0.70b    |
| ASMg$^{2+}$| 5 mg/kg (17)   | 5.40±0.27    | 4.93±0.43     |
| ASMg$^{2+}$| 10 mg/kg (18)  | 6.24±0.38a   | 5.05±0.15f    |
| ASMg$^{2+}$| 20 mg/kg (19)  | 8.48±0.33c   | 5.30±0.23b    |
| ASLi$^+$  | 5 mg/kg (20)   | 7.64±0.37c   | 6.28±0.24     |
| ASLi$^+$  | 10 mg/kg (21)  | 7.37±0.48b   | 6.28±0.24     |
| ASLi$^+$  | 20 mg/kg (22)  | 4.38±0.61l   | 5.30±0.31     |

a Significance of differences compared to the control group (p≤0.05).  
b Significance of differences compared to the control group (p≤0.01).   
c Significance of differences compared to the control group (p≤0.001).  
d Significance of differences compared to group 2 (p≤0.05).   
e Significance of differences compared to group 3 (p≤0.05).  
f Significance of differences compared to group 3 (p≤0.001).  
g Significance of differences compared to group 4 (p≤0.05).  
h Significance of differences compared to group 4 (p≤0.01).  
i Significance of differences compared to group 4 (p≤0.001).
ASCo$^{2+}$ at a dose of 20 mg/kg increased TFL by 107% (p<0.05) and HPTL by 81.7% (p<0.05) in relation to these parameters in the control group of animals (Table 1). In other doses, ASCo$^{2+}$ did not significantly change the values of these parameters.

In the “tail-flick” test, ASNi$^{2+}$ increased TFL at a dose of 5 mg/kg by 35.19% (p<0.05) and at a dose of 20 mg/kg – by 35.00% (p<0.05) relative to the control (Table 1). In the “hot plate” test, a significant increase in HPTL was observed when ASNi$^{2+}$ was administered at a dose of 10 mg/kg – by 62.14% (p<0.05), and at a dose of 20 mg/kg – by 66.17% (p<0.05) relative to the values in the control group (Table 1).

In the “tail-flick” test, ASZn$^{2+}$ only at a dose of 10 mg/kg significantly increased TFL by 45.6% (p<0.05) relative to the control values (Table 1). In the “hot plate” test at all doses, as well as in the “tail-flick” test at doses of 10 and 20 mg/kg under the influence of ASZn$^{2+}$, HPTL did not significantly change.

In the “tail-flick” test, ASMn$^{2+}$ at doses of 5, 10 and 20 mg/kg in rats significantly increased TFL by 45.6% (p<0.05), 122.3% (p<0.001) and 77.5% (p<0.001), respectively, relative to the control (Table 1). In the “hot plate” test, only at a dose of 20 mg/kg ASMn$^{2+}$ (Table 1) expressed in a significant increase in HPTL by 47.6% (p<0.01) relative to the control. This indicates an analgesic effect of ASMn$^{2+}$ at a dose of 20 mg/kg.

In the “tail-flick” test, ASMg$^{2+}$ significantly increased TFL by 39.2% (p<0.05) and by 89.3% (p<0.001) at doses of 10 and 20 mg/kg (Table 1), respectively, relative to the control. In the “hot plate” test, ASMg$^{2+}$ in all doses did not significantly change HPTL (Table 1).

In the “tail-flick” test, ASLi$^{+}$ significantly increased TFL by 70.5% (p<0.001) and 64.5% (p<0.01) at doses of 5 and 10 mg/kg, respectively, relative to those in the control group (Table 1). In the “hot plate” test, ASLi$^{+}$ at all studied doses did not significantly change HPTL (Table 1).

Thus, ASA and metal salicylates increase the thresholds of PS in male rats. This demonstrates the presence of analgesic properties in these compounds, depending on the dose of the compounds and the pain stimulus used.

It is known [8-11] that an increase TFL indicates the involvement of the perceptual component and the spinal mechanism of PS regulation, and an increase HPTL indicates the influence on the supraspinal mechanisms of PS. Based on this, it can be concluded that ASA and ASCo$^{2+}$, ASNi$^{2+}$, ASZn$^{2+}$, ASMn$^{2+}$, ASMg$^{2+}$ and ASLi$^{+}$ in different doses have an analgesic effect with the participation of various mechanisms of pain regulation.

The analysis of the “chemical structure-properties” relationship, which in this case is a reflection of AS compounds with metals biological action effectiveness in comparison with the ASA, showed that the introduction of metals into the ASA structure reduces the analgesic effect of newly synthesized salicylates in comparison with ASA. This is evidenced by the significantly low values of the pain sensitivity thresholds presented in Table 1 and EC (Figure 1 and 2), calculated as the ratio of the effect of the corresponding AS with metal to the effect of ASA. As it can be seen from Figures 1 and 2, almost all the performance indicators of the tested AS metals are in the negative region of the histogram, which indicates a lower analgesic effect of the compounds compared to ASA. The exception is ASMn$^{2+}$, whose analgesic effect at a dose of 10 mg/kg exceeds that of ASA, however, the significance of this effect is not reliable (Table 1). It is likely that the presence of analgesic properties in ASCo$^{2+}$, ASNi$^{2+}$, ASZn$^{2+}$, ASMn$^{2+}$, ASMg$^{2+}$ and ASLi$^{+}$ is due to the presence of salicylic acid in the tested compounds.

It can be concluded that the introduction of complexing metals in ASA molecules reduces their analgesic properties, and such a design of compounds is not advisable when creating highly active analgesics.
4. Conclusion
1. ASA in all doses had a pronounced analgesic effect with the participation of spinal and/or supraspinal mechanisms of PS regulation. This is evidenced by a significant increase in the PS thresholds in the tests of thermal acute pain “tail-flick” and “hot plate”.

2. The analgesic effects ASNi^{2+}, ASMn^{2+} and ASLi^{+} were manifested when this compounds were administered in all three doses, ASMn^{2+} – at doses of 10 and 20 mg/kg, ASZn^{2+} – only at a dose of 10 mg/kg, ASCo^{2+} – only at a dose of 20 mg/kg.

3. The introduction of metal-complexing agents in ASA molecules reduces their analgesic properties, and the design of connections is not appropriate when creating potent analgesics.
5. References

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Acknowledgements
The study was carried out with the financial support of the RFBR in the framework of scientific project No. 20-33-70142 on the experimental equipment of the Center for Collective Use of Scientific Equipment “Experimental Physiology and Biophysics” of the Department of Human and Animal Physiology and Biophysics of the Taurida Academy (structural division) V. I. Vernadsky Crimean Federal University.