



SYSTEMICALLY ADMINISTERED COBALT – PHARMACOLOGICAL DATA REGARDING AN ANTINOCICEPTIVE ACTION

B. I. Tamba, Irina Jaba, Dunărea Ionescu, O. C. Mungiu

Department of Pharmacology-Algesiology, Center for the Study and Therapy of Pain, “Gr. T. Popa” University of Medicine and Pharmacy Iași

Abstract. Aim of investigation. Cobalt (Co) is an essential divalent trace element. Because Zinc and Magnesium (also divalent trace elements) are well known for their influence on the nociceptive processes, we looked upon the possible modulator effect in nociception after systemically administered Co.

Methods. Groups of 7 mice were treated with Cobalt Chloride (3.75 mg/kg body weight), administered intraperitoneal. Different tests were used for evaluating the antinociceptive effect or the influence on behavior of the tested substances: thermal nociception (hot plate test, tail flick test), chemical nociception (writhing test) and spontaneous behavior (activity cage assay).

Results. Our preliminary data for response latencies for hot-plate and tail-flick tests suggest that systemically administered Co produces a significant analgesic effect under thermal nociceptive stimulation. The spontaneous behavior assay also shows a significant decrease of activity in the tested animals.

Discussion. Pain inhibition is even more significant in conditions of chemical nociceptive stimulation, in a model of visceral pain.

Conclusion. The mechanism through which Co exerts its analgesic effect is still unclear, and will require more investigations, including dose-effect analysis, though it may be related to the influence of haeme oxygenase-1 on the inflammatory pain pathways.

Keywords: Cobalt, nociception, analgesia

Introduction

Cobalt is necessary as a trace element for all cells but is toxic at high concentrations, a fact of considerable environmental importance. It is the central metal cofactor in the corrin ring of vitamin B₁₂ and also plays crucial roles in biological functions.

The transmission of pain signals at spinal level

is crucially dependent on the activation of Ca²⁺ channels in nociceptive neurons. The subsequent neuronal membrane depolarization is essential for nociceptive afferent inputs transmission, pain integration and associated sensitization at spinal and thalamic nociceptive neurons' level. [2]

The divalent cations cobalt, magnesium and manganese all block synaptic transmission without blocking conduction in fibers-of-passage. They do so by blocking calcium channels, thereby preventing calcium uptake at the presynaptic terminal and subsequent release of synaptic transmitter. [1,4,5,9,10,11,14,15]

Having this background as starting point, the present paper is trying to identify more arguments for a Cobalt role in nociception.

Ostin C. Mungiu, professor
Department of Pharmacology-Algesiology,
Center for the Study and Therapy of Pain,
“Gr. T. Popa” University of Medicine and Pharmacy
Iași, Romania
Email: ocmungiu@yahoo.com

Materials and methods

Animals. All experimental procedures employed in the present study were strictly in accordance with the international guidelines regarding ethics. The animal breeding facility of the Central Drug Testing Laboratory, "Gr. T. Popa" University of Medicine and Pharmacy, Iași supplied male Swiss-Webster mice with an average weight of 20g (\pm 2g). The animals were housed in a temperature-controlled room ($21^{\circ}\text{C} \pm 2^{\circ}\text{C}$) with a 12 hours/12 hours light/dark cycle, 4 mice per cage, and were allowed to acclimate for at least 24 hours before use, with free access to food and water.

Drug administration. Groups of 7 mice were treated with Cobalt (Co) chloride (Sigma-Aldrich Chemie GmbH) 3.75 mg/kg body weight, 0.1 ml solution administered intraperitoneal. The control group received an equal volume of saline solution.

Analgesic tests. Different tests were used for evaluating the antinociceptive effect or the influence on behavior of the tested substances: thermal nociception (hot plate test, tail flick test), chemical nociception (writhing test) and spontaneous behavior (activity cage assay).

For the **tail flick latency test**, animals were placed inside restraining cages at least 5 min before tail flick latency determination. Constant heat intensity was applied to the dorsum of the lower third of the animal's tail and when the animal flicked its tail in response to the noxious thermal stimulus, both the heat source and the timer were automatically stopped. A maximum tail flick latency of 15 sec was permitted to minimize tissue damage to the mouse's tail. The test was performed at 15, 30, 45 and 60 minutes after the administration of substances or saline (control).

For the **hot-plate latency test**, a rectangular metal surface was heated to a temperature of $55 \pm 0.5^{\circ}\text{C}$. The antinociceptive response was the latency observed from the time the mouse was placed on the heated surface until the first overt behavioral sign of nociception such as (i) the mouse licking a hind paw, (ii) vocalization, or (iii) an escape response. The timer was stopped by a foot-operated pedal and the rat was immediately removed from the hotplate. A maximum hotplate latency of 60 seconds was used to prevent tissue damage to the mouse's paws. The nociceptive response was the latency observed from the moment the animal was placed on the heated surface until the first overt behavioral sign of nociception. Hot-plate test was

performed at 15, 30, 45, and 60 minutes after the administration of substances or saline (control).

Treatments that produced a significant increase in the latency response in hot plate or tail flick tests were considered to be antinociceptive.

The abdominal stretch, or **writhing assay**, was performed by injecting 0.1 ml of 1.0 % acetic acid intraperitoneal, in manually restrained mice. Immediately after injection, animals were placed in a large glass cylinder. The number of abdominal stretches occurring in successive 5 minutes time intervals was counted starting at 5 minutes after acetic acid, during a 30 minutes period after intraperitoneal injection of diluted acetic acid. Co was administered 5 minutes prior to the acetic acid intraperitoneal injection. Values are reported as the mean (\pm S.E.M.) for each treatment with groups composed of seven mice. Hand-operated counters and stopwatches were employed to score writhing frequency of the mice placed in glass cages. Treatments that produced a significant decrease in the number of abdominal stretches were considered to be antinociceptive. The mice were kept under observation for a period of 72 hours and then sacrificed.

Activity cage assay. This test is performed in order to record spontaneous coordinate activity in mice (individual) and variation of this activity in time. Mice were transported to the testing room the night before testing. Animals were weighed and tested between 9:00 and 11:00 AM. Horizontal and vertical locomotor **activity** was monitored for 2 minutes with the Ugo Basile Activity Cage System. The system is represented by an I.R. Beam Array Cage, consisting in a cubicle of clear Perspex: two facing blocks containing an I.R. array record the horizontal activity. A similar System assesses the vertical activity (rearing). Horizontal and vertical **activity** was defined as the total number of beam interruptions throughout a 2 minutes observation period. The test was performed 15 minutes following the administration of Co.

Data analysis. Statistical analysis of the results was performed using the one-way ANOVA test. The p values of under 0.05 were used for indicating a significant difference for all tests. Mean values of the test groups were routinely compared with control values collected the same day.

Results and discussion

The nociceptive evaluation secondary to Co (3.75 mg/kg b.w.) intraperitoneal administration

has shown a statistically significant increase in response latencies for tail flick thermal nociceptive stimulation. This antinociceptive effect reached its peak at 15 minutes and lasted for a time span of 30 minutes. ($p < 0.05$) (Figure 1).

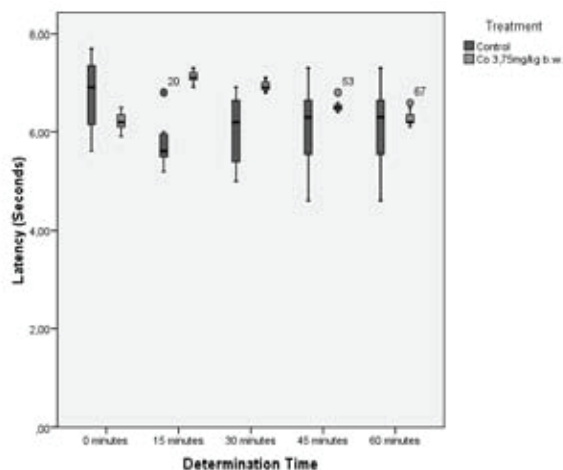


Figure 1 Tail Flick testing

The intraperitoneal administration of Co (3.75 mg/kg b.w) also induced a mild and transient increase in latencies to nociceptive thermal stimuli in mice tested for hot plate test. The peak values were reached at 15 minutes consequently to Co administration. The significant increase in response latency was recorded at 60 minutes after the administration. (Figure 2)

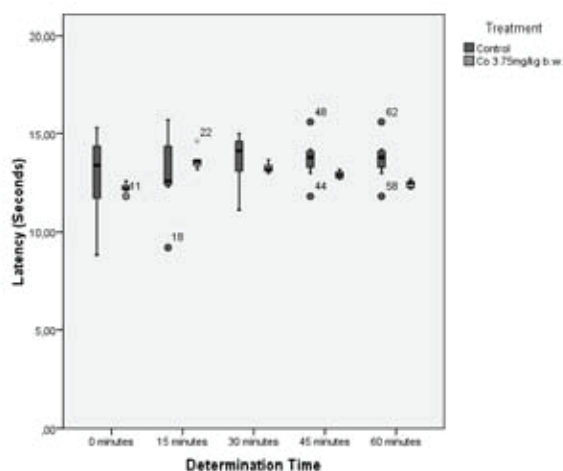


Figure 2 Hot plate testing

Co administration consequently to chemical nociception induced with acetic acid (writhing test) is followed by a rapid decrease in the number of

abdominal stretches. The effect has immediate onset – 10 minutes after Co treatment and 5 minutes consequently to nociceptive intraperitoneal irritation with acetic acid, and lasts at highly significant levels ($p < 0.001$) of analgesic inhibition for the 30 minutes time span after acetic acid administration. For toxicology reasons the mice were kept under strict observation for a period of 72 hours after the testing. No further manifestations have been noted, and no fatality was recorded. (Figure 3)

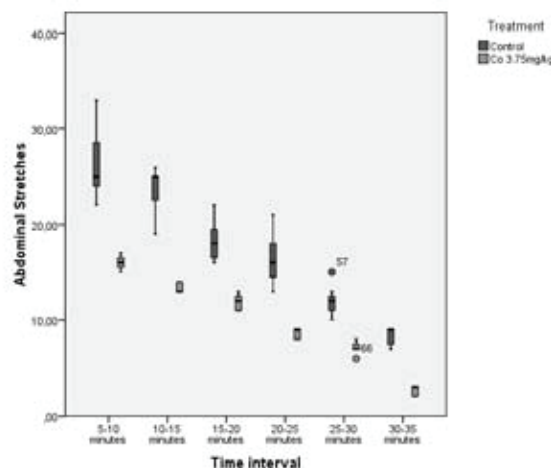


Figure 3. Writhing Test

When tested with activity cage assay secondary to Co treatment, the observed mice proved a significant decrease of spontaneous activity both on the vertical and horizontal axes. (Figure 4).

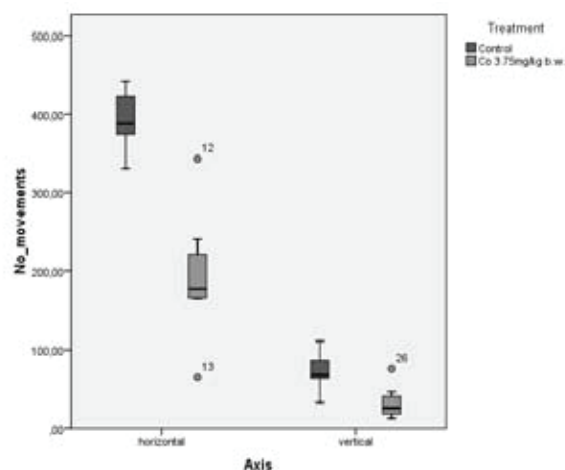


Figure 4. Activity cage testing

Divalent cations, including Co, share biologically important chemical properties with the divalent cal-

cium (Ca) cation and are known to block Ca channels. However, a possible role on pain processing and transmission has not been directly investigated. A recent study has pointed that haeme-oxygenase-1 (HO-1) modulates the inflammatory pain pathways with Co, as part of a cobalt protoporphyrin, which is an HO-1 inducer. In another unrelated study conducted on hypotension, Co was shown to have an analgesic effect, though no mechanism was presented. [3,7,8,11,13]

In this context, the significant decrease of spontaneous activity in the tested animals could indicate a depressant action of cobalt. Therefore a possible analgesic effect by means of antidepressant mechanisms might be involved.

The analgesic effect observed secondary to Co treatment in this research is to be confirmed by further studies, as there are only a couple of published works on the matter. Still, more detailed studies targeting the mechanism of Co antinociceptive activity are necessary. [6]

Conclusions

Values for response latencies obtained for hot-plate and tail-flick tests prove that Co administered systemically produces an analgesic effect under thermal nociceptive stimulation. This action is especially strong shortly after Co treatment.

Pain inhibition is even more significant in conditions of chemical nociceptive stimulation, in a model of visceral pain. In these circumstances the analgesic activity of Co appears much earlier consequently to drug administration [8], or by means of a mechanism like the blockade of the calcium channels or the HO-1 influence on the inflammatory pain, as shown by recent research.

Acknowledgements: Work supported by the Ministry of Education and Science grant CNCSIS 31GR./2007 and the “Physiopharmacological and clinical studies platform on oncologic and nononcologic pain” no. 68

References

1. **Abdel-Azim Assi** - The Influence Of Divalent Cations On The Analgesic Effect Of Opioid And Non-Opioid Drugs, *Pharmacological Research*, 2001;43(6).

2. **Angstadt JD, Friesen WO** - Synchronized oscillatory activity in leech neurons induced by calcium channel blockers., *J Neurophysiol.* 1991;66(6):1858-73.

3. **Dehuang Guo, Jennifer Ling, Mong-Heng Wang, Jin-Xiong She, Jianguo Gu, and Cong-Yi Wang** - Physical interaction and functional coupling between ACDP4 and the intracellular ion chaperone COX11, an implication of the role of ACDP4 in essential metal ion transport and homeostasis, *Mol Pain.* 2005; 1: 15. Published online 2005 April 19. doi: 10.1186/1744-8069-1-15.

4. **Frederickson CJ** - Neurobiology of zinc and zinc-containing neurons, *Int. Rev. Neurobiol.*, 1989;31:145-238

5. **Giniatullin R, Sokolova E, Nistri A** - Modulation of P2X3 receptors by Magnesium²⁺ on rat DRG neurons in culture. *Neuropharmacology.* 2003;44(1):132-40.

6. **Hamann SR, Holtman JR, Martin WR** - Analgesic actions of local anesthetics and cobalt chloride in the rat brain stem. *Pharmacol Biochem Behav.* 1992;43(3):925-7.

7. **Hideobu Komeda, Michihiko Kobayashi, Sakayu Shimizu** - A novel transporter involved in cobalt uptake, *Proc. Natl. Acad. Sci. USA, Applied Biological Sciences* 1997;94:36-41.

8. **Joseph G. Malpeli** - Reversible inactivation of subcortical sites by drug injection, *Journal of Neuroscience Methods.* 1999;86:119-128

9. **Kara H, Sahin N, Ulsan V, Aydogdu T** - Magnesium infusion reduces perioperative pain. *European Journal of Anaesthesiology* 2002;19:52-6

10. **Larson AA, Kelley KF** - Manipulations of zinc in the spinal cord, by intrathecal injection of zinc chloride, disodium-calcium-EDTA, or dipicolinic acid, alter nociceptive activity in mice, *J Pharmacol Exp Ther*, 1997;282:1319-1325

11. **Prado WA, Machado Filho EB** - Antinociceptive potency of aminoglycoside antibiotics and magnesium chloride: a comparative study on models of phasic and incisional pain in rats. *Braz J Med Biol Res.* 2002;35(3):395-403.

12. **Rosa AO, Egea J, Lorrio S, Rojo AI, Cuadrado A, López MG** - Nrf2-mediated haeme oxygenase-1 up-regulation induced by cobalt protoporphyrin has antinociceptive effects against inflammatory pain in the formalin test in mice. *Pain.* 2007;25

13. **Sinan Cavun, Gokhan Goktalay and Millington WR** - The hypotension evoked by visceral nociception is mediated by delta opioid receptors in the periaqueductal gray, *Brain Research*, 2004;1019,1-2, 3:237-245

14. **Tramer MR, Schneider J, Marti RA, Rifat K** - Role of magnesium sulfate in postoperative analgesia. *Anesthesiology* 1996;84(2):340-7.

15. **Weiss JH, Sensi SL, Koh JY** - Zn²⁺: a novel ionic mediator of neural injury in brain disease, *Trends Pharmacol Sci*, 2000;21:395-401