# Digoxine reduces thermal pain threshold and neuromuscular coordination in rats

***Digoxine reduce el umbral del dolor térmico y la coordinación neuromuscular en ratas***

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## Abstract

It is well known that inhibition of Na,K-ATPase by digoxine induces an increased $[Na^+]_i$ and $[Ca^{++}]_i$ with consequent increased cell depolarization. This effect has a potential implication on nociception. To analyze this digoxine effect on pain threshold and neuromuscular activity, 20 male Sprague-Dawley rats (~300gr) were treated with NaCl 1mL, 0.9% (Controls, n=10) or digoxine 1mL, 40 $\mu g.Kg^{-1}.day^{-1}$ (Digoxine, n=10) i.p. for a week. Daily test was performed for pain threshold by hot plate test (50±0.1ºC, mean±SEM) and neuromuscular motor coordination by rotarod test (at 17 rpm). Digoxine reduced 28% the hot plate latency (17.16±2.05s) when compared to controls (23.83±2.32s; P<0,001) and reduces neuromuscular activity in 95.4% (1.02±5.42s digoxine vs 22.22±5.50s; P<0.001). Both effects were observed from the first doses. There was no correlation neither between hotplate and rotarod tests latencies nor between these values and the accumulative doses of digoxine. These results strongly suggest the pronociceptive effect of digoxine by decrease of the thermal pain threshold and that this could be more intense than reported due to the masking effect of a reduced motor activity during behavioral tests. The present results contribute to explain recent report of increased pain in digoxine treated human patients.

**Key Words**: Digoxine, Na,K-ATPase, Pain threshold, Hot plate test, Rotarod test.

## Resumen

Es bien conocido que la inhibición de la Na,K-ATPasa por la digoxina induce un incremento en el $[Na^+]_i$ y $[Ca^{++}]_i$ con la consecuente depolarización celular. Este efecto posee una potencial acción en nocicepción. Para analizar este efecto sobre el umbral del dolor y sobre la actividad neuromuscular, se trataron 20 ratas macho adultas Sprague-Dawley (~300g) con NaCl 1mL, 0,9% i.p. (Controles, n=10) o digoxina 1mL, 40 $\mu g.Kg^{-1}.dia^{-1}$ i.p. (Digoxina, n=10) por una semana. Diariamente se determinaron el umbral del dolor (hot plate test 50±0,1ºC; media±EEM) y coordinación neuromuscular (rotarod test a 17rpm). La digoxina redujo 28% la latencia del test hot plate (17,16±2,05s) al compararlo con los controles (23,83±2,32s; P<0,001) y redujo la actividad neuromuscular en 95,4% (1,02±5,42s digoxina vs 22,22±5,50s; P<0,001). Ambos efectos se observaron a partir de la primera dosis de digoxina. No se detectó correlación ni entre las latencias del hot plate test y rotarod test ni entre estos valores y la dosis acumulada de digoxina. Estos resultados sugieren un efecto pronociceptivo de la digoxina al reducir el umbral al dolor térmico, efecto que puede ser aún mas intenso que el reportado debido a la acción enmascaradora de la reducción de la actividad motora durante las pruebas conductuales. El presente estudio contribuye a explicar recientes reportes de dolor incrementado en pacientes tratados con digoxina.

**Palabras Clave**: Digoxina, Na,K-ATPasa, Umbral del dolor, Hot plate test, Rotarod test.

## Introduction

Membrane ATPases are widely expressed at pain processing areas of the spinal cord[1] and have been associated to pain and inflammation processes with a potential antinociceptive effect. Acute peripheral inflammation increases not only Na,K-ATPase but also Na-ATPase[2] and fluoride resistant acid phosphatase[3] in the ipsilateral spinal dorsal horn. The consequences of this increased activity is to restore the $Na^+$ and $K^+$ gradients associated to continued neuronal discharges[2,3] and to reduce the glutamate release[4]. These factors are strongly associated with hyperalgesia and allodynia[5,6]. Digoxine, a potent Na,K-ATPase inhibitor, increases the intracellular concentration of sodium inducing neuronal depolarization and consequently the voltage dependent calcium influx[2,7] thus, promoting neuronal excitability which was associated to inflammation[2] and even postulated such as a potential cause of increased pain in humans[8]. In mice, digoxine was able to antagonize the antinociceptive effect of morphine in mice[9].

Different antinociceptive mechanisms have been described in the central nervous system such as diffuse nociceptive inhibitory control (DNIC), propriospinal antinociceptive responses and descending modulatory system[5,6,10]. Each of these mechanisms includes both excitatory and inhibitory neurons within their circuits, but in all of them the ATPase activity is present. This fact makes difficult to estimate the resulting response after the ATPase inhibitory action of digoxine.

Despite of a profuse cardiovascular clinical use and detailed studies about basic mechanisms of action of digoxine, less attention has been paid to the potential pain sensation changes induced by this ATPase inhibitor. Spinally-applied ouabain, another Na,K-ATPase inhibitor, showed contradictory results, i.e., antinociception[11], no effect[12] in rat tail flick test or anti-inflammatory effect in mice[13]. These controversies require more investigation under different controlled laboratory conditions to analyze an integrative behavioral response, i.e., sensory and motor aspects, to noxious stimuli.

## Materials and Methods

The experimental procedures were carried out according to the guide for the care and use of laboratory animals of the National Institute of Health and the U.S. Public Health Office on the use of experimentation animals[14] (NIH 1996) and the protocol was approved by the Dirección de Investigación y Producción Intelectual, Facultad de Ciencias de la Salud, Universidad de Carabobo. Male Sprague-Dawley rats (~300gr.) housed in 5 animals per cage were maintained with food and water *ad libitum* in a light:dark, 12:12 hours (lights on at 06:00h) schedule in a temperature-controlled (26±2°C, mean±SEM) environment. Rats were daily habituated to the testing room and to test devices, i.e., to hot plate at environment temperature and trained to walk on the rotating rotarod device for at least one week before any test commenced.

### Drug treatment

Rats were treated daily at 8:00 am to avoid circadian changes in ATPase activity[15]. A 1mL NaCl 0.9% was administered via i.p. (Controls, n=10) or digoxine (Novartis™) 1mL at 40 $\mu g.Kg^{-1}.day^{-1}$ (n=10) in random order within groups, drug treatment for 7 days. Used dose is between reported ranges applied to rats (10 to 200 $\mu g.Kg^{-1}.day^{-1}$)[16,17]. Daily test started 1 hour after drug administration with a random selection of the rats and serial and random hot plate and rotarod tests.

### Hot plate test

Pain threshold was measured by hot plate test[18] at 50±0.1°C to test mainly supraspinal generated response[19]. Once placed on the device, the latency was measured by a stopwatch until the animal displays a total of 3 of any nocifencive responses like jumping, licking a paw or vocalizing[18]. The cutoff latency was set at 30s. Immediately previous to the hot plate test, each rat was let to stabilize their foot temperature putting them on an electric blanket at 35°C for 3 minutes; this achieves the same foot thermal gradient for all rats at the moment of the hotplate test[20]. Both groups of rats were tested the day before the beginning of the treatment.

### Rotarod test

The rotarod test set at a fixed rotational speed of 17 rpm, was applied to assess sensorimotor coordination[21]. The latency to fall off of the rotarod was measured by a stopwatch. A cutoff latency of 200 s was used for all rotarod assessments. Both groups of rats were tested the day before the beginning of the treatment. Every rat was tested 4 times a day for both hot plate and rotarod tests during 7 days and from the 09:00 to14:00 hours.

### Statistics

Latencies from hot plate and rotarod tests were expressed as mean ± SEM, non parametrical Wilcoxon test was applied for group comparison, Pearson correlation analysis was used to find association between hot plate and rotarod values. Statistical significance was set at $P<0.05$. PAST v2.04 statistical software[22] was used.

## Results

Hot Plate test

Hot plate latency (280 readings for each group) of digoxine group (17.16±2.05s) were significantly shorter (27.98%) than those from the control group (23.83±2.32s; $z=5.51$; $P<0.001$). Figure 1 presents the daily time course of the mean hot plate latency for each group of rats during the 7 days of treatment, it is noted that differences between groups were statistical significant since the first day i.e., 24 hour after the beginning of digoxine treatment.

Correlation analysis did not detects association between daily hot plate latency and day of the treatment neither for control ($r=-0.14$; $P>0.05$) nor digoxine group ($r=-0.15$; $P>0.05$).

### Rotarod test

Digoxine induced a reduction of the motor activity of the rats within their cages. This was also observed with the rotarod test latency since the first test day, i.e., 24 hours after the beginning of treatment, digoxine group latency (1.02±5.42s) were 95.36% shorter than those from the control group (22.22±5.50s; z=5.43; P<0.001). Figure 2 presents the daily rotarod latency for each group, where differences between both groups take place for every test day.

Correlation analysis did not detects association between daily rotarod latency and day of the treatment neither for control (r=-0.18; P>0.05) nor digoxine group (r=0.29; P>0.05). Hot plate and rotarod latency did not show statistical significant association neither in control (r=0.57; P>0.05) nor in digoxine group (r=-0.36; P>0.05).

**Figure 1. Pain Threshold expressed by the mean±SEM of hot plate latency (s) for every day of the control (saline; open squares) and digoxine (40 µg.Kg-1.day-1) treated rat groups (solid squares).**

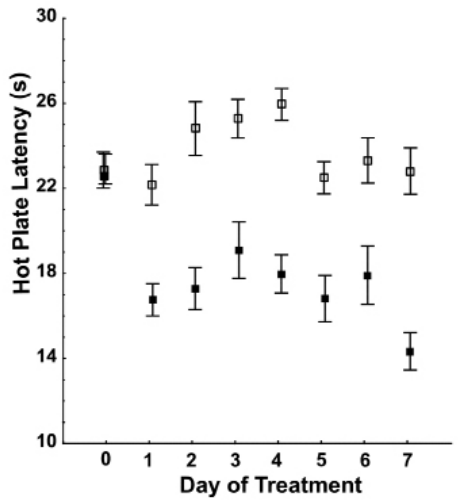
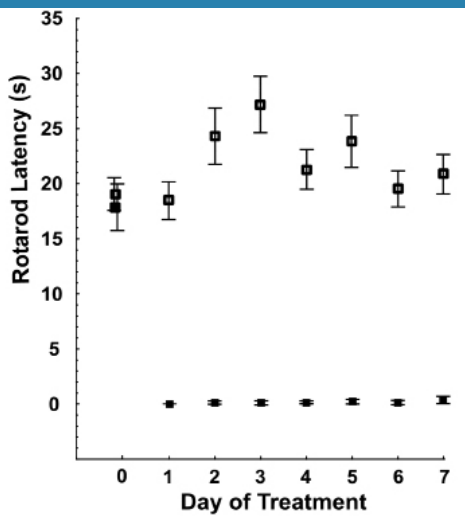



**Figure 2. Sensorimotor coordination expressed by the mean±SEM of rotarod latency (s) for every day of the control (open squares, saline) and digoxine (solid squares, 40µg.Kg-1.day-1) treated rat groups.**




## Discusion

In the present study, it was found in rats that digoxine reduces 28% the pain threshold and 95% the sensorimotor coordination since the application of the first doses of 40 µg.Kg$^{-1}$.day$^{-1}$. Recent report has shown an increase in the subjective pain sensation taking place in patients receiving digoxine as a part of their cardiovascular treatment[8], however, due to their cardiovascular disease, i.e., heart failure, these patients should receive combinations of another drugs, i.e., β-blockers and diuretics that generate doubts about the actual effects of digoxine on nociception. Laboratory controlled conditions of the present study strongly support the notion that digoxine really increases the pain sensation by lowering the thermal pain threshold.

The $\beta_3$ subunit of the Na,K-ATPase mediates variable nociceptive sensitivity in the acute phase of the formalin test[23] which evaluates direct stimulation and early nociceptor responses, and further predicts that manipulations that decrease the gene Atp1b3 expression for this subunit, and/or pump functioning would increase pain sensitivity. These facts agree with our results in the way that inhibition of the ATPase activity increases thermal pain sensibility by lowering threshold.

The intense reduction in neuromuscular motor coordination detected since the first day of digoxine treatment, suggests that the induced lowering in pain threshold could be greater than herein reported 28% because this value was obtained during and despite the strong reduction in the sensorimotor coordination which is part of the behavioral response of the hot plate test. Ataxia inducing effect measured on the rotarod test, was also reported in mice experiments with ouabain, another Na,K-ATPase inhibitor, at doses that modify early phase of the formalin test[23]. Intrathecal administration of digoxine in humans[24] and rabbits[25] have been reported to induce paraesthesias, paralysis and lower limbs reflexes due to a dose dependent $Na^+$ pump inhibition.

Direct involvements of ATPases activities in nociceptive centers have been reported in the last years, Czaplinsky et al.,[2] demonstrated that a nociceptive stimuli like heat-induced inflammation led to an increase of the Na,K- and Na-ATPase activity in spinal dorsal horn, supporting the notion that the increased neuronal excitability increases sodium and calcium influxes and thus, stimulating ATPase activity aimed to reduce these influxes and to achieve neuronal repolarization.

Consistently, well known analgesic substances like morphine and encephaline analogues significantly increase Na,K-ATPase activity, on the other hand, the opiate antagonist, naloxone, decreased the activity of Na,K-ATPase[26]. Additionally, intracerebroventricular application of ouabain antagonizes opioid analgesia which suggests its effect on supraspinal Na,K-ATPase[9].

The digoxine induced inhibition of the Na,K-ATPase in neurons, leads to an increase of the intracellular sodium concen-

trations which depolarizes the neuron, increases their excitability[2,4,7] and opens voltage sensitive calcium and sodium channels with a strong neuronal depolarization[5,6,10]. However, GABA/Glycine containing interneurons in the spinal dorsal horn tonically inhibit nociceptive transmission[10]. These neurons could also be excitated by Na/K-ATPase inhibition exerting antinociception rather than pronociception. Ionic pump membrane densities, inhibitor selectivity, inhibition time course, dose, spinal or extra spinal neuronal location differences must be considerated to explain the imbalance toward pronociception reported here for a clinical like administered digoxine which effect was similar than those reported for a human study[8].

The results obtained by the hotplate test at 50ºC used in the present study must be linked to low intensity pain sensation due to reports that at different test temperature difference channels could be activated such as the case for Acid-sensing ion channels (ASICs) for 52.5 and 55 vs. 50°C[18].

The present report supports the interesting antinociceptive role of ATPases by evaluation of their inhibition; further studies of effects of its stimulation, pointing out a potential analgesic effect are needed.

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