Beta-1 adrenoceptor blockade decreases the firing rate to painful stimuli in spinal wide-dynamic range neurons in rats

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Los bloqueadores β-1 adrenérgicos reducen la respuesta a estímulos dolorosos en neuronas de amplio rango dinámico de la médula espinal en ratas

Introducción: la epinefrina/norepinefrina inhibe la transmisión del dolor agudo; empero, no es claro el papel de los receptores β-adrenérgicos. Por tanto, analizamos si los fármacos de estos receptores modulan la transmisión del dolor agudo mediante registro electrofisiológico unitario extracelular *in vivo* durante estimulación periférica dolorosa y no dolorosa en ratas.
Métodos: estudio longitudinal en el que se cotejaron siete grupos de ratas: control (*n* = 11): solución salina (0,9 %); EPI (*n* = 8): 100 mcg epinefrina; AGOβ1 (*n* = 8): 125 mcg dobutamina; ANTβ1 (n = 9): 100 mcg metoprolol; AGOβ2 (*n* = 7): 100 mcg clembuterol; ANTβ2 (n = 8): butoxamina 100 mcg; ANTβ1 + EPI (*n* = 10): 100 mcg metoprolol + 100 mcg epinefrina. Se hizo análisis estadístico por medio de ANOVA.
Resultados: La epinefrina redujo significativamente la tasa de disparo basal (RDB) en 34.1 % ($p < 0.05$) y la respuesta evocada por la estimulación dolorosa en 56 % ($p < 0.05$). No hubo cambios en la respuesta provocada por la falta de estimulación dolorosa. El ANTβ1 fue el único fármaco con acción β-adrenérgica que redujo significativamente la respuesta evocada por la estimulación dolorosa en 41 % ($p < 0.05$).
Conclusión: por primera vez un antagonista de los receptores β1-adrenérgicos (metoprolol) prueba ser eficaz en la reducción de la respuesta a la estimulación dolorosa en las neuronas ARD.

Keywords	Palabras clave
Receptors, Adrenergic	Receptores adrenérgicos
Acute pain	Dolor agudo
Metoprolol	Metoprolol
Beta-Blockers, Adrenergic	Bloqueadores beta adrenérgicos

One of the most studied phenomena regarding acute pain modulation is stress-induced analgesia (SIA). SIA is an in-built mammalian pain suppression response that occurs following or during exposure to a stressful or fearful stimulus.[1] Many different mechanisms mediate SIA; it mainly occurs through activation of the sympathetic nervous system, and produces a release of norepinephrine (NE) within the central nervous system;[1] besides NE, many other molecules are involved in this process, but in a lesser extent (e.g. endogenous opioid, cannabinoids and γ-aminobutyric acid).[2,3,4]

Even though many brain areas have been implicated in SIA, e.g. amygdala,[5] or periaqueductal gray,[6] the spinal cord is thought to be the first gate to acute pain perception[7] and, thus, where most of the regulation occurs, particularly at wide dynamic range (WDR) neurons.[8] These neurons are considered WDR, because they are polymodal, and respond to somatosensory as well as nociceptive inputs.[8] Moreover, the aforementioned descending inhibitory pathways play a role in SIA by inhibiting these neurons.[9,10]

It is known that epinephrine and norepinephrine mediate analgesia in the spinal cord.[11,12,13,14] Adrenoceptors are divided into three families: α1, α2 and β. Alpha 1 and β are excitatory receptors whereas α2 are inhibitory receptors.[15] Since the latter are inhibitory, the majority of studies have focused on the role of these receptors in pain modulation, suggesting that SIA is only mediated by activation of α2-adrenoreceptors expressed in sensory neurons.[12,13,14]

From all the subtypes of β-adrenoceptors, β1 and β2 subtypes are the most expressed within the central nervous system (CNS).[15,16] Even though there is sparse, non-conclusive evidence that β-adrenoceptors are implicated in pain modulation in animal models,[17,18,19] there is a broad spectrum of clinical pain states (fibromyalgia,[20] migraine,[21] and also in painful phantom limb) which improve after taking β-adrenergic antagonists.[22] Furthermore in patients with untreatable oncological pain, labetalol (non-selective β-adrenoceptor antagonist) relieved pain

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Abstract

Background: It is known that epinephrine/norepinephrine inhibit acute pain transmission. However, the role of β-adrenoceptors is not clear. Thus, we analyzed if β-1 and/or β-2 adrenoceptors can modulate acute pain transmission by performing *in vivo* single unit recordings during painful and non-painful peripheral stimulation in rats.

Methods: Longitudinal study in which we analyzed seven groups of male rats Wistar: control group (n = 11): saline (0.9 %); EPI group (n = 8): epinephrine 100 mcg; AGOβ1 group (n = 8): dobutamine 125 mcg; ANTβ1 group (n = 9): metoprolol 100 mcg; AGOβ2 group (n = 7): clenbuterol 100 mcg; ANTβ2 group (n = 8): butoxamine 100 mcg; ANTβ1 + EPI group (n = 10): metoprolol 100 mcg + epinephrine 100 mcg. For the statistical analysis we used ANOVA.

Results: Epinephrine significantly reduced the basal firing rate (BFR) in 34.1 % ($p < 0.05$) and also the evoked response by painful stimulation in 56 % ($p < 0.05$). No change was observed in the evoked response by non-painful stimulation. ANTβ1 was the only β-adrenoceptor acting drug that significantly reduced the evoked response by painful stimulation in 41 % ($p < 0.05$). None of the other drugs alone affected either the BFR or the evoked response to non-painful or painful stimulation.

Conclusion: It is the first time that a β1-adrenoceptor antagonist (metoprolol) probes to be effective in reducing the response to painful stimulation in WDR neurons.

in 40 % of the patients treated. More recently, other authors reported that β2-adrenoceptors are implicated in the antihyperalgesic effect of non-selective serotonin-norepinephrine reuptake inhibitors as it is shown in a mouse model of neuropathic pain.[23,17]

To our knowledge the role of β-adrenoceptors in acute pain modulation has not yet been fully studied, so the aim of this work is to address their role by studying the pattern of activity of spinal WDR neurons in response to non-painful and painful stimulation in animals treated with agonists and antagonists of β-adrenoceptors.

Methods

Experiments were performed in male Wistar rats (250-350 g). The Ethics Committee of the Instituto Nacional de Psiquiatría "Ramón de la Fuente Muñiz" approved all the procedures, and the experiment was under the regulatory guidelines of the International Association for the Study of Pain (IASP) for animal studies.[24] Rats were housed and were cared for in the institute's animal facility until the day of the experiment. They were kept in groups of five per cage with food and water *ad libitum*. All surgical procedures and recordings were performed at room temperature (between 25 and 27°C).

Surgical procedures

Rats were anesthetized with an intraperitoneal injection of urethane (1.3 mg/kg), an anteroposterior medial incision was performed on the skin, muscles were retracted and a bilateral dissection was performed under L2-L4 in order to introduce the stereotaxic spinal cord holders and keep the vertebrae fixed (figure 1). Afterwards, we removed the laminae from L2 and L3 as well as the meningeal tissue.

Electrode positioning, receptive field and neuron characterization

Tungsten electrodes (70 mm, 3 MΩ, FHC Inc. Bowdoin, ME) were used to record extracellular *in vivo* single unit responses from WDR neurons from the spinal cord. To place the electrode we used a manual hydraulic positiometer (KOPF Instruments, model 640, Tujunga, California). To record sensory neurons of the right hind paw of the animal, the electrode was placed on the right side of the dorsal spinal cord at L3 level. We descended the electrode from the spinal cord surface to a maximum depth of 500 μm while we applied a non-painful mechanical stimulation until the receptive field was found. WDR neurons were considered to be those responsive to both painful and non-painful stimuli; hence, once the receptive field was found a non-noxious painful stimulus was applied in order to characterize the neuron as wide-dynamic or as a non-painful sensory neuron. Only WDR neurons were included in the study.

Electrophysiological recording and data analysis

For each animal, the same stimuli sequence (SS) was performed twice, separated by a 300 s stimuli-free interval. The complete experiment scheme is depicted in figure 1.

Cerebus system (Cyberkinects INC, USA) was used for the amplification of the signal. All neurons were characterized as WDR neurons with Spikesorter (version 1.0.0.1). Neuroexplorer (version 4.097) was used to analyze the data. The entire 520 s recording was split into 10 s histograms, which correlate to the duration of each interval. From the complete length of the recording, we only analyzed the first and the last 90 s, corresponding to the SS before drug administration and to the SS after drug administration (figure 1). The number of spikes detected for each kind of

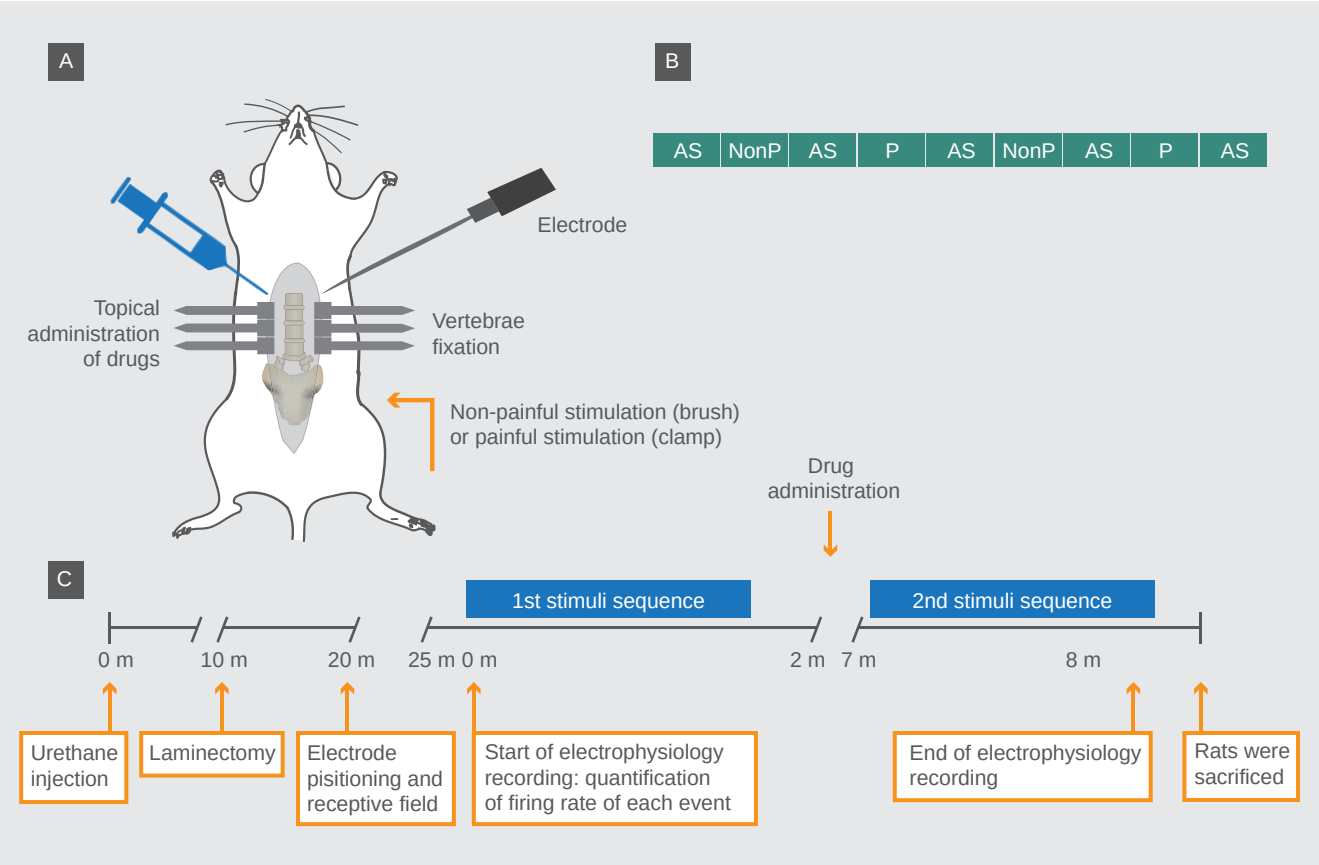

Figure 1 Process to introduce the stereotaxic spinal cord holders in male Wistar rats

interval was averaged, obtaining three different values from each SS: 1) average from five AS-intervals, 2) average from two Non-P-intervals, and 3) average from two P-intervals, vide supra. Data obtained for each type of interval were converted to percentage values, where 100 % of response corresponded to the average of spikes in all the AS-intervals during the first SS for each neuron analyzed. We defined this as the basal firing rate (BFR). The effect of the drug on the firing rate was calculated by comparing the firing rate before and after drug administration for each type of stimuli to the BFR, as well as comparing the firing rate evoked by each type of stimuli to its reciprocate in the second SS. Therefore, we observed either an increase or a decrease in the percentage of BFR and in the firing rate evoked P stimuli and Non-P stimuli.

Mechanical stimulation

Two different types of stimuli were applied to the right hind paw during the recording: Non-painful (Non-P) mechanical stimuli with a brush, painful (P) mechanical stimuli with a clamp. The duration of each stimulus was 10 s. Each P and Non-P stimuli were alternated and separated by a 10 s interval where no stimulus was applied (absence of stimulation, AS) in order to quantify the basal firing rate of each neuron. The SS was divided into intervals as follows: AS – NonP – AS – P – AS – NonP – AS. The duration of the stimuli sequence was 90 s.

Statistical analysis

One-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test were used to analyze the results, and a p value < 0.05 was considered to represent a significant difference.

Groups and drug administration

The vehicle for all drugs was saline at 0.9 %. Drugs were administered topically on the spinal cord (volume: 100 µl). Experimental details for each group are depicted in table 1.

Results

A total of fifty-three spinal WDR neurons were analyzed in our study. Results will be presented in per-

Table I Experimental details for each group of rats

Group	*n*	Drug	Dose	Mechanism of action
Control	11	Saline at 0.9 %	---	---
EPI	8	Epinephrine	100 mcg	Complete adrenoreceptor agonist
AGOβ1	8	Dobutamine	125 mcg	β1-adrenoreceptor agonist
ANTβ1	9	Metoprolol	100 mcg	β1-adrenoreceptor antagonist
AGOβ2	7	Clenbuterol	100 mcg	B2-adrenoreceptor agonist
ANTβ2	8	Butoxamine	100 mcg	B2-adrenoreceptor antagonist

centage values, indicating a decrease or increase in either the basal firing rate or the firing rate evoked by Non-P stimuli and P stimuli produced under the adrenoceptor-acting drugs application.

Effects of adrenoceptor-acting drugs on the BFR

Figure 2 shows changes in BFR obtained by the comparison of the average of all the AS-intervals from first SS with all the AS-intervals from the second SS. In the control group (n = 11) we observed that the BFR was higher (an increase of 27.49 %) during the second SS without any drug present; however, this increase was non-significant. Thus, we decided to compare data from all the drug-tested groups with this difference.

Complete adrenergic agonist administration (epinephrine, EPI group) produced a significant decrease ($p < 0.05$) in the BFR of –34.1 %. None of the beta-adrenoceptor drugs significantly changed the BFR ($p > 0.05$); the results were the following: β1-adrenoceptor agonist (dobutamine, AGOβ1 group) produced a decrease in the BFR of –0.05 %, β2-adrenoceptor agonist (clenbuterol, AGOβ2 group) produced a minimum change of 0.52 %, β1-adrenoceptor antagonist (metoprolol, ANTβ1 group) produced an increase of 10.56 % whereas β2-adrenoceptor antagonist (butoxamine, ANTβ2 group) produced an increase of 4 %.

Adrenoceptor-acting drugs and the evoked response to non-painful stimulation

Changes in the firing rate evoked by non-painful stimuli were obtained by comparison of the average of the two Non-P intervals from the first SS with the average of the two Non-P intervals from the second SS.

In the control group (n = 11) we observed a reduction (–5.97 %) in the firing rate evoked by non-painful stimulation; however, it was non-significant. All drug-tested groups were compared to this group. No significant change was observed in the control group or any of the drug-tested groups (ANOVA $p > 0.05$). Results were as following: In the EPI group we observed a change of –0.05 %, in the AGOβ1 group a change of 8.73 %, in the ANTβ1 group a change of –23 %, in the AGOβ2 group a change of 9.63 %, and, finally, in the ANTβ2 group we observed a change of 20.62 % (figure 3).

Adrenoceptor-acting drugs and the evoked response to painful stimulation

Changes in the firing rate evoked by painful stimuli were obtained by the comparison of the average of the two P intervals from the first SS with the average of the two P intervals from the second SS. They are shown in figure 3.

In the control group we observed a reduction (–1.18 %) in the firing rate evoked by painful stimulation; however, it was non-significant. All drug-tested

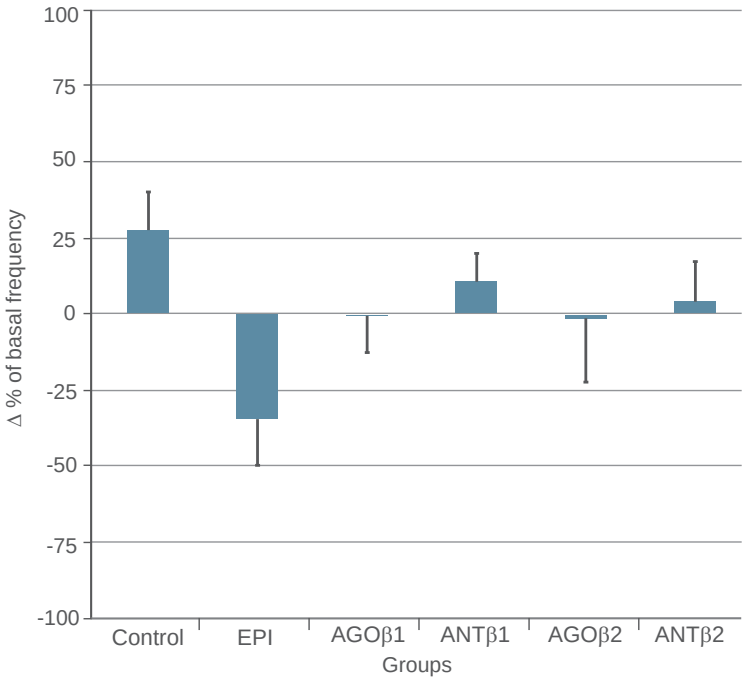

Figure 2 Changes in basal firing rate (BFR) by comparing all the AS-intervals from the second stimuli sequence

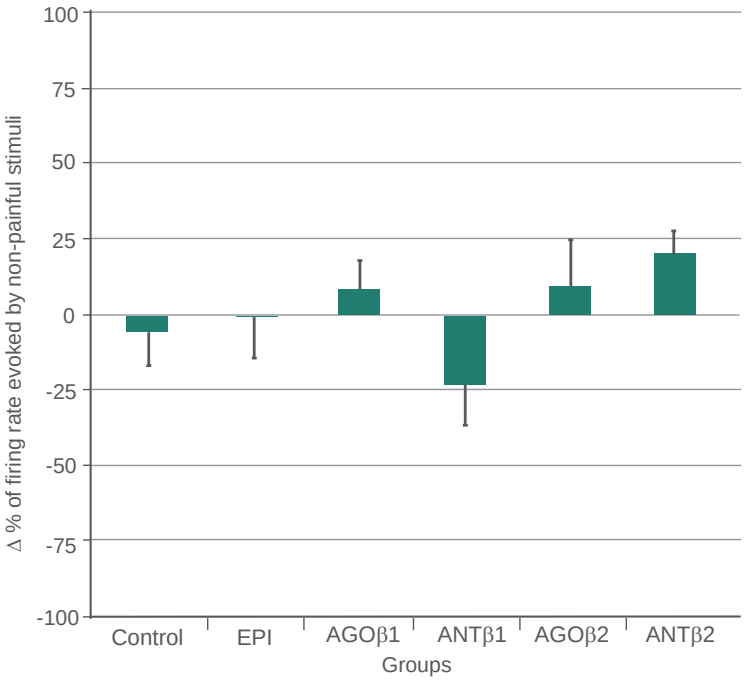

Figure 3 Changes in the firing rate evoked by non-painful stimuli

groups were compared to this group. We observed reduction in the firing rate evoked by painful stimulation of –56.6 %. This difference was significantly different when compared to the control group (ANOVA, post-hoc LSD, $p < 0.05$). In the ANTβ1 group we observed a significant reduction in the firing rate of –41 % (ANOVA, post-hoc LSD, $p < 0.05$). None of the other β-adrenoceptor agents significantly changed the firing rate evoked by painful stimulation (ANOVA, post-hoc LSD, $p > 0.05$). In the AGOβ2 we observed a change of –26 %, in the AGOβ1 group a change of 12 %, and, finally, in the ANTβ2 group a change of 3.5 % (figure 4).

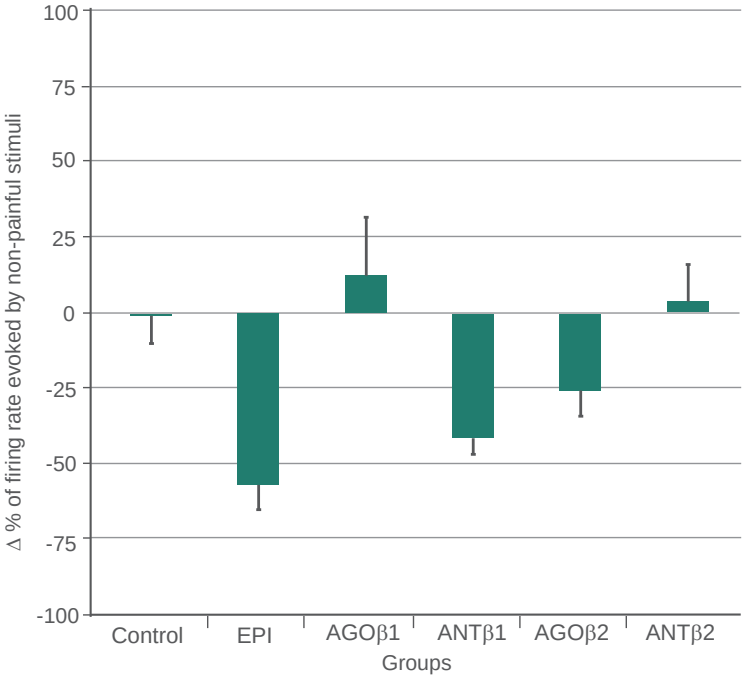

Figure 4 Changes in the firing rate evoked by painful stimuli

Effects on BFR and firing rate evoked by non-painful and painful stimuli after EPI+ANTβ1 co-administration

From the 10 neurons analyzed, seven neurons (70 %) showed a significant decrease in either, the BFR and in the evoked response to painful stimulation, whereas three of the neurons did not change significantly their BFR or the evoked response to any type of stimuli. Changes in the firing rate were obtained in the same manner than the rest of the groups. Here we show the results of the responsive neurons. Figure 5 shows the changes in the BFR as well as the changes in the firing rate evoked by non-painful and painful stimulation in presence of co-administration of EPI + ANTβ1 and compared to the control group. EPI + ANTβ1 reduced the BFR in –37.9 % and the firing rate evoked by painful stimuli in –47.81 %. Both changes were significant when compared to the control group (ANOVA < 0.05, post-hoc LSD, $p < 0.05$). This combination of drugs did not change significantly (–19 %, $p > 0.05$) the evoked response to non-painful stimulation.

Discussion

In the present study we evaluated the role of adrenoceptors in acute pain modulation by observing the effect that different adrenoceptor-acting agents have on the firing rate evoked by painful/non-painful stimulation, as well as analyzing the basal firing rate (BFR) of spinal WDR neurons.

Some *in vivo* experiments have reported that after painful peripheral stimulation spinal WDR neurons increase their BFR,[25] but it has also been documented that painful peripheral stimulation can trigger inhibitory descending mechanisms resulting in a decrease of the BFR of these neurons.[26,27] The increase in the BFR observed in the control group after painful stimulation supports the former phenomenon. Also, the fact that the differences in the firing rate evoked by non-painful and painful stimuli observed in the control group were stable and non-significant may imply that the changes seen in the drug-tested groups were produced by the drugs and not by descending inhibitory mechanisms.

From all the β-adrenergic-acting agents, metoprolol (β1-antagonist) significantly diminished the

response of spinal WDR neurons to painful peripheral stimulation whereas β2-adrenoceptor antagonist did not affect in any way the firing rate of these neurons.

To our knowledge, β1-adrenoceptors have never been implicated in pain perception; nevertheless, labetalol (non-selective β-adrenoceptor antagonist) enhances the effect of morphine but not necessarily the physiological response to peripheral painful stimulation.[18] In humans there are reports of non-selective β-adrenoceptor antagonist to treat[28,20,22] or prevent[21] pain in these conditions. Our results imply that β1-adrenoceptor may be involved in normal acute pain transmission, since the blockade of this receptor produces a decreased response to painful peripheral stimulation.

Given that metoprolol produces a decrease in the firing rate evoked by painful stimuli, but does not affect the BFR, in addition to the fact that dobutamine (a β1-adrenoceptor agonist) does not increase the activity of either, these receptors may be changing the activity of nociceptive-specific neurons and not that of WDR neurons. The fact that spinal WDR neurons have inputs from non-nociceptive and nociceptive sensory neurons supports these hypothesis, although more inmunohistochemical studies regarding the expression of adrenoceptors in these neurons are needed to support this evidence.

In our hands, neither clenbuterol (β2adrenoceptor-agonist) nor butoxamine (β2adrenoceptor-antagonist) showed a significant difference in the activity of WDR neurons, although there are some interesting results in neuropathic pain models that the activation of β2-adrenoceptors has shown to play a role in the mechanisms underlying the antihyperalgesic effect of norepinephrine/serotonin reuptake.[17,23] Even though non-selective β-adrenoceptor antagonists have analgesic properties in certain pain conditions,[20,19,21] our results point that this effect may be limited to β1-adrenoceptors.

The lack of effect of clenbuterol may be to the fact that the density of the β2 subtype in neurons is not as abundant as the β1 subtype as reported by Nicholas *et al*.[29] In this work they show that β1-adrenoceptors are expressed mainly in neurons whereas β2-adrenoceptors are expressed mainly in glial cells.

In agreement with other studies,[1,9,11,32,10,34,30] our results show that epinephrine decreased the response of WDR neurons to painful stimulation. Moreover, the marked reduction in the basal firing rate (BFR) seen in the EPI-tested group suggests that epinephrine has not only analgesic effects, but also has in some extent an anesthetic effect. Also, our results support the idea that stress-induced analgesia is mediated by spinal WDR neurons, given by the fact that epinephrine administration reduced the firing rate evoked by painful stimuli.

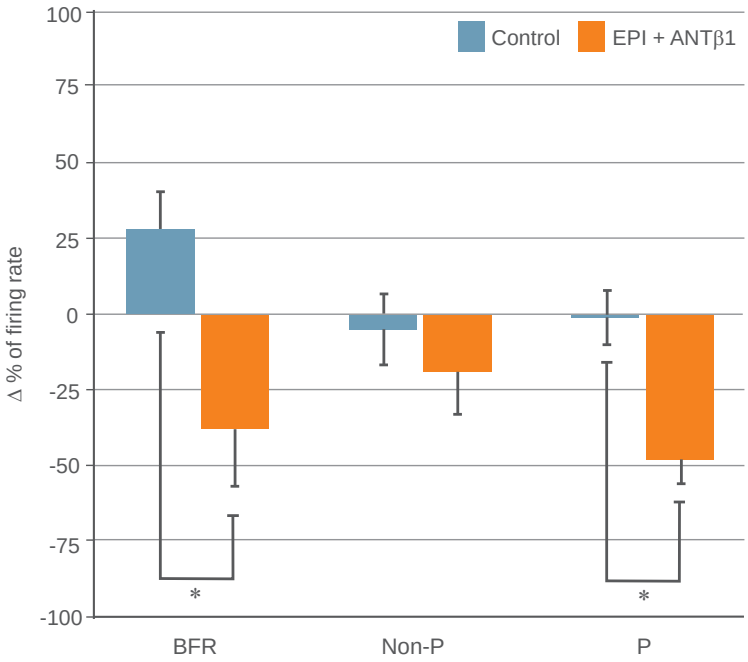

Figure 5 Changes in the basal firing rate and in the rate evoked by non-painful and painful stimuli

It is noteworthy that neither AGOβ1 nor AGOβ2 alone altered significantly the activity of WDR neurons, which implies that the acute pain modulation of epinephrine could be, as it is widely reported in the literature, due to the activation of an α2-adrenoceptor. Another hypothesis is that this analgesic effect is mediated by activation of all the subtypes of adrenoceptors and not exclusively by one subtype, as it is seen in other non-neural tissues.[31]

The results observed in the ANTβ1-EPI support the latter hypothesis; on the one hand, the effect of epinephrine was not enhanced by metoprolol, as seen by the fact that the decrease in the activity of WDR neurons seen in this group was not significantly different than the one observed in the EPI-tested group or the one observed in the ANTβ1 group.

On the other hand, we did not find a homogeneous response in all neurons, only 70 % of the neurons significantly decreased their activity while the other 30 % of the neurons increased their activity in a non-significant manner. This indicates that interactions of greater complexity dictate the activity of WDR neurons in the spinal cord. As a result of this a reductionist approach such as this one is insufficient to fully describe the effects of the adrenergic system in pain transmission; systemic approaches will surely shed more light upon the matter. Further research, including patch-clamp studies, may be needed to understand the role of these receptors on acute pain modulation.

Limitations of the study

Even though we only registered spinal WDR neurons, the drugs were administered topically on the spinal cord, hence the drugs tested may have also affected other types of neurons (e.g. nociceptive-specific, GABAergic interneurons, etcetera) involved in pain modulation.

Conclusions

WDR neurons are an important site of pain regulation[32,27] because of the diverse modulatory and sensitive inputs they receive, making them a more attractive site for pain modulation study than nociceptive-specific neurons.

Collectively, our results show that adrenoceptors modulate acute pain transmission by affecting the activity of spinal WDR neurons, but they do not modify non-painful sensory transmission. The analgesic effects seen with some non-selective β-adrenoceptor antagonists may be mainly due to β1-adrenoceptors expressed in nociceptive-specific neurons, but not in spinal WDR. Although α2-adrenoceptors are an actual target for pain treatment, our results suggest that β1-adrenoceptors could be a new pharmacological target for acute pain.

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Conflicts of interest: All of the authors have filled and sent the translated-to-Spanish form of the declaration of potential conflicts of interest of the International Committee of Medical Journal Editors, and it was not reported any conflict with regards to this article.

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