

# Effects of oxytocin and oxytocin receptor antagonist atosiban on nociception and morphine analgesia in rats

## Sıçanlarda oksitosin ve oksitosin reseptör antagonisti atosibanın nosisepsiyon ve morfin analjezi üzerine etkileri

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### SUMMARY

**Objective:** Oxytocin is a peptide-based hormone released from the supraoptic nucleus and paraventricular nucleus in the hypothalamus and consisting of nine amino acids. It has been shown that oxytocin may have an effect on opiate receptors. Atosiban is a oxytocin receptor antagonist. Our aim in this study was to investigate the effects of oxytocin and atosiban on nociception and morphine analgesia.

**Method:** In our study, 48 Wistar Albino 230-260 g male rats were used. The animals were divided into eight groups (control, 200 µg/kg oxytocin, 3 mg/kg atosiban, oxytocin+ atosiban, 5 mg/kg morphine, morphine+ oxytocin, morphine+atosiban and morphine+ oxytocin+ atosiban). Serum physiologic to the control group, oxytocin and atosiban were given intraperitoneally (i.p.) at the indicated doses to the other groups. Morphine was administered subcutaneously (s.c.). Analgesic effects were assessed by hot plate and tail flick analgesia tests. The resulting analgesic effect was measured and recorded at the 15th, 30th, 60th, 90th and 120th minutes. Assessment of analgesic effect was formulated as % analgesia (MPE) (% analgesia = 100x [post drug reaction time-pre drug reaction time]/ [cut off time-pre drug reaction time]).

**Results:** Oxytocin showed analgesic activity (p<0,05). Atosiban showed hyperalgesic activity and decreased the analgesic activity of oxytocin when given with oxytocin (p<0,05). Oxytocin increased the analgesic activity of morphine (p<0.05). In addition, although atosiban did not alter the analgesic activity of morphine, morphine analgesia increased by oxytocin was reduced (p<0.05).

**Conclusions:** According to these results, it can be said that although atosiban does not change on the analgesic effect of morphine alone, it blocks the effect of oxytocin on morphine analgesia.

**Keywords:** Atosiban, oxytocin, nociception, morphine analgesia, analgesia tests

### ÖZET

**Amaç:** Oksitosin, hipotalamusta supraoptik çekirdek ve paraventrikülerçekirdeklerden salınan, dokuz amino asitten oluşan, peptid yapılı bir hormondur. Oksitosin'in opiyat reseptörleri üzerinde etkili olabileceği gösterilmiştir. Atosiban, oksitosinin reseptör antagonistidir. Bu çalışmada amacımız, oksitosin ve atosibanın nosisepsiyon ve morfin analjezi üzerine etkilerini araştırmaktır.

**Yöntem:** Çalışmamızda 48 Wistar Albino 230-260 g erkek sıçan kullanıldı. Hayvanlar kontrol, 200 µg/kg oksitosin, 3 mg/kg atosiban, oksitosin+atosiban, 5 mg/kg morfin, morfin+oksitosin, morfin+ atosiban ve morfin+oksitosin+atosiban olmak üzere sekiz gruba ayrıldı. Kontrol grubuna serum fizyolojik, diğer gruplara belirtilen dozlarda oksitosin ve atosiban intraperitoneal (i.p.) olarak verildi. Morfin, subkütan (s.c.) yoldan verildi. Analjezik etkinliktail flick ve hot plate analjezi

testleriyle değerlendirildi. Ortaya çıkan analjezik etki 15., 30., 60., 90. ve 120. dakikalarda ölçüldü ve kaydedildi. Analjezik etkinin değerlendirilmesi % analjezi (MPE) (% analjezi =  $100 \times [\text{ilaç sonrası tepki süresi} - \text{ilaç öncesi tepki süresi}] / [\text{test kesme süresi} - \text{ilaç öncesi tepki süresi}]$ ) şeklinde formüle edildi.

**Bulgular:** Oksitosin analjezik etkinlik gösterdi ( $p < 0,05$ ). Atosiban, hiperanaljezik aktivite gösterdi ve oksitosin ile birlikte verildiğinde oksitosin'in analjezik etkinliğini azalttı ( $p < 0,05$ ). Oksitosin morfinin analjezik etkinliğini arttırdı ( $p < 0,05$ ). Buna ek olarak, atosiban morfinin analjezik etkinliğini değiştirmese de, oksitosin ile artmış morfin analjezisini azalttı ( $p < 0,05$ ).

**Sonuç:** Bu sonuçlara göre, atosibanın tek başına morfinin analjezik etkisini değiştirmemesine rağmen, oksitosinin morfin analjezi üzerindeki etkisini bloke ettiği söylenebilir.

**Anahtar sözcükler:** Atosiban, oksitosin, nosisepsiyon, morfin analjezi, analjezi testleri

## INTRODUCTION

Oxytocin (OT) is a mammalian neurohypophysial hormone that synthesized in the hypothalamic paraventricular nucleus and supraoptic nucleus<sup>1</sup>. Oxytocinergic neurons display widespread projections through the central nervous system (CNS). Oxytocin receptors also widely distribute in the CNS, including hypothalamus, thalamus, olfactory system, cortex, and dorsal horn in the spinal cord<sup>2</sup>. OT has several hormonal functions such as the regulation of parturition and lactation by respectively facilitating uterine contraction and milk ejection<sup>3</sup>. In addition to these peripheral functions, it acts as a neuromodulator a variety of brain functions such as learning and memory<sup>4</sup>.

Oxytocin was showed the anti-nociceptive effect in experimental animals intraperitoneally and intracisternally. In addition to, when oxytocin injected into periaqueductal gray matter and raphe Magnus nucleus remarkable decreased nociceptive response in rats<sup>5</sup>. Different physiological functions of oxytocin done via its receptor that coupled to GTP binding proteins (Gq/11) which stimulate together with G $\beta\gamma$  the activity of phospholipase C- $\beta$  isoforms<sup>6</sup>. Being a dezamino-OT analogue, atosiban is a nonapeptide, and is a vasopressin/OT receptor antagonist. Atosiban inhibits the release of inositol triphosphate, realized via OT from myometrial cell membrane<sup>7</sup>.

Although some studies indicated that there are interaction opioid system and oxytocin receptors, this was unclear and limited<sup>8, 9</sup>. Our aim in this study was to investigate the effects of oxytocin receptor antagonist atosiban on oxytocin anti-nociceptive effect and morphine analgesia.

## MATERIAL AND METHODS

### Animals

This study was conducted at Cumhuriyet University University Experimental Research

Center with the permission granted by Local Animal Studies Ethical Board (CUHEK/2014-67). In this study were used a total of 66 adult male Wistar albino rats (230-250 g). The rats were housed four per cage in a room maintained at  $21 \pm 2$  °C with an alternating 12 hour dark-12 hour light cycles and free access to water and food. Experimental animals were acclimatized to laboratory conditions before the analgesia test. All experiments were carried out blindly between 10:00 and 17:00 h.

### Antinociceptive tests

To evaluate thermal nociception, it was used a standardised Tail Flick (TF) test (May TF 0703 Tail-flick Unit, Commat) and Hot Plate (HP) test (May AHP 0603 Analgesic Hot-plate Commat, Turkey). In TF test, the radiant heat source was focused on the distal portion of the tail at 3cm after administration of the vehicle and study drugs. Following vehicle or compound administration, tail-flick latencies (TFL) were obtained. The infrared intensity was adjusted so that basal TFL occurred at  $2.9 \pm 0.5$  s. Animals with a baseline TFL below 2.4 or above 3.4 s were excluded from further testing. The cutoff latency was set at 15 s to avoid tissue damage. Any animal not responding after 15 s was excluded from the study. The analgesic response in this test is usually attributed to central mechanisms<sup>10</sup>.

In HP test, the rats were individually placed on a HP with the temperature adjusted to  $55 \pm 0.5$  °C. The latency to the first sign of paw licking or jump response to avoid the heat was taken as an index of the pain threshold; the cut-off time was 30 s in order to avoid damage to the paw. The antinociceptive response on the HP test is considered to result from a combination of peripheral and central mechanisms<sup>11</sup>.

### Drugs

In this experiment, oxytocin, atosiban and morphine were used (Sigma Co., USA).

## Experimental protocols

48 male 230-260 g Wistar Albino rat were divided into 8 groups as follows; control, oxytocin (40IU/kg), atosiban (3mg/kg), oxytocin+atosiban, morphine (5mg/KG), morphine+oxytocin, morphine+atosiban and morphine+oxytocin+atosiban. Oxytocin and atosiban were injected intraperitoneally (i.p.). Morphine was injected subcutaneously (s.c.). After injections, analgesic effects were assessed by hot plate and tail flick analgesia tests. The resulting analgesic effect was measured and recorded at the 15th, 30th, 60th, 90th and 120th minutes.

## Data analysis

In order to calculate % maximal antinociceptive effects (% MPE), lick/escape latencies (hot-plate) and tail-withdrawal latencies (tail-flick) were converted to percent antinociceptive effect using the following equation:

$$\% \text{ MPE} = [( \text{test latency} - \text{baseline} ) / ( \text{cutoff} - \text{baseline} )] \times 100$$

## Statistical analysis

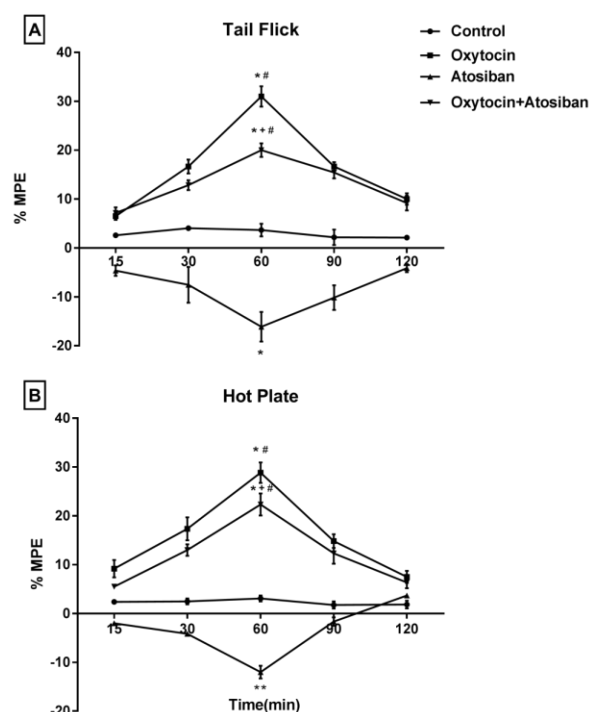
The data (% MPE) were analysed by two-way analysis of variance (ANOVA) and repeated measures ANOVA followed by a Tukey post hoc

test for multiple comparisons between groups (SPSS 20.0 for windows). All data are presented as means  $\pm$  S.E.M. The level of significance was set at  $p < 0.05$ .

## RESULTS

### Effects of atosiban and oxytocin on nociception

The analgesic activity of oxytocin (tail-flick:  $16,16 \pm 1,31$  and hot-plate:  $15,53 \pm 1,08$ ), and oxytocin-atosiban (tail-flick:  $12,91 \pm 1,31$  and hot-plate:  $11,9 \pm 1,08$ ) groups were significantly higher than control group (tail-flick:  $3,66 \pm 3,18$  and hot-plate:  $2,31 \pm 1,08$ ) in both the tail-flick ( $p < 0.05$ ; Fig. 1A) and hot-plate ( $p < 0.05$ ; Fig. 1B) assays. The analgesic activity of atosiban (tail-flick:  $-8,52 \pm 1,31$  and hot-plate:  $-3,23 \pm 1,08$ ) group were significantly lower than control group rats in both the tail-flick ( $p < 0.05$ ; Fig. 1A) and hot-plate ( $p < 0.05$ ; Fig. 1B). When oxytocin and atosiban are given together, atosiban decrease oxytocin analgesic activity in both the tail-flick ( $p < 0.05$ ; Fig. 1A) and hot-plate ( $p < 0.05$ ; Fig. 1B) assays. In addition, The analgesic activity of oxytocin and oxytocin-atosiban groups significantly higher than atosiban group in both the tail-flick ( $p < 0.05$ ; Fig. 1A) and hot-plate ( $p < 0.05$ ; Fig. 1B) assays.

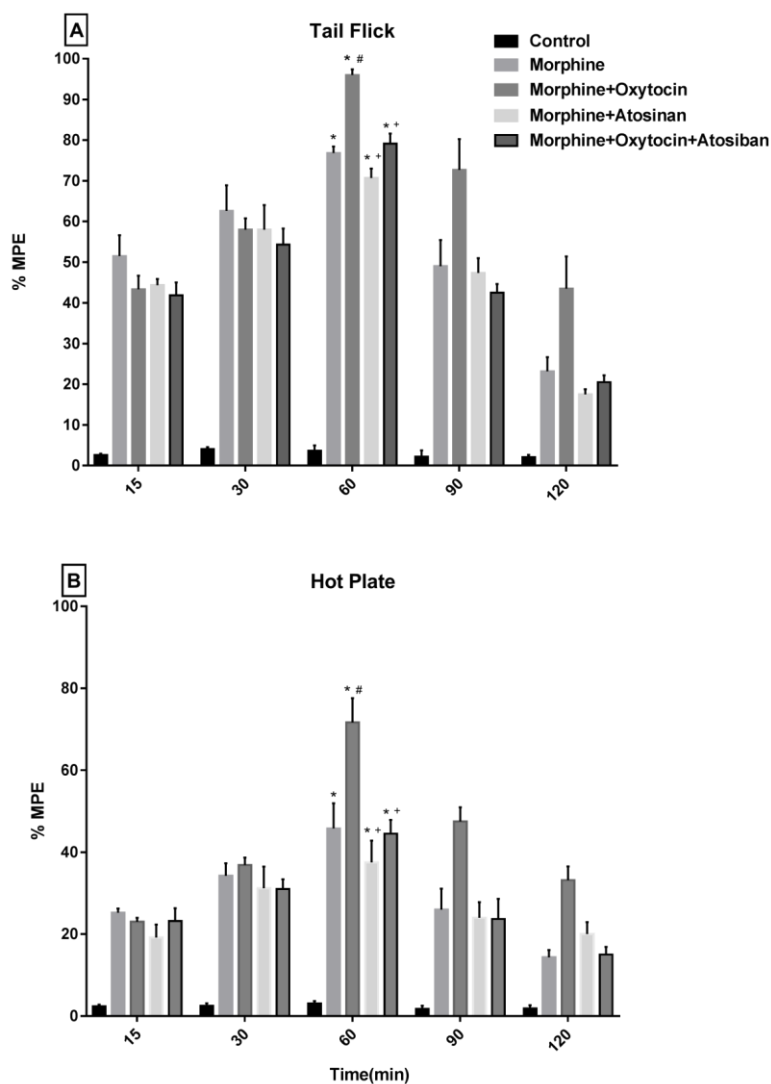


**Figure 1.** Effects of oxytocin and atosiban on nociception. (A) shows effect of oxytocin and atosiban in the tail-flick test; (B) shows effect of oxytocin and atosiban in the hot-plate test. Each point represents the mean  $\pm$  SEM of % MPE for 6 rats. \* $p < 0.01$  compared to the control, \*\* $p < 0,05$  compared to the control group, + $p < 0,05$  compared to oxytocin group the # $p < 0.01$  compared to the atosiban group.

### Effect of atosiban and oxytocin on morphine analgesia

Obtained data suggested that administration of morphine (5 mg/kg; tail-flick:  $76,80 \pm 1,57$  and hot-plate:  $45,78 \pm 6,14$ ) produce a significant increase in % MPE in both the tail-flick ( $p < 0.05$ ; Fig. 2A) and hot-plate ( $p < 0.05$ ; Fig. 2B) assays as compared to saline group rats (tail-flick:  $3,66 \pm 1,30$  and hot-plate:  $3,08 \pm 0,59$ ). The analgesic activity of morphine significantly increased when it given oxytocin (tail-flick:  $96,00 \pm 1,39$  and hot-plate:  $71,$

$66 \pm 5,9$ ) together in both the tail-flick ( $p < 0.05$ ; Fig. 2A) and hot-plate ( $p < 0.05$ ; Fig. 2B) assays. Moreover, The analgesic activity of morphine did not change when it given atosiban together (tail-flick:  $70,66 \pm 2,33$  and hot-plate:  $37,5 \pm 5,32$ ) in both the tail-flick ( $p > 0.05$ ; Fig. 2A) and hot-plate ( $p > 0.05$ ; Fig. 2B) assays. However, when oxytocin and atosiban given with morphine, increased morphine analgesic activity with oxytocin decreased (tail-flick:  $79,16 \pm 2,44$  and hot-plate:  $44,50 \pm 3,34$ ) in both the tail-flick ( $p < 0.05$ ; Fig. 2A) and hot-plate ( $p < 0.05$ ; Fig. 2B) assays.



**Figure 2.** Effects of oxytocin and atosiban on morphine analgesia. (A) shows effect of oxytocin and atosiban on morphine analgesia in the tail-flick test; (B) shows effect of oxytocin and atosiban on morphine analgesia in the hot-plate test. Each point represents the mean  $\pm$  SEM of % MPE for 6 rats. \* $p < 0.01$  compared to the control group, # $p < 0.01$  compared to the morphine group and + $p < 0.01$  compared to morphine+oxytocin group.

### DISCUSSION

Results of several studies have shown that oxytocin has analgesic effect<sup>12</sup> and part of analgesic effect of that is related to inhibition of spinal glutamatergic

transmission<sup>13</sup>. One of the possible mechanisms for the analgesic effect of oxytocin in normal animals is the action of oxytocin on its G protein receptor which leads to metabolization of intracellular

calcium and subsequently a nonspecific cation channel open and potassium channel close<sup>14, 15</sup>. It was reported that oxytocin can block glutamate activation and causing decreased nociceptive responses in spinal cord cells<sup>16</sup>. It was also demonstrated that in acute and chronic recurrent pain a significant change is occurred in content of oxytocin in human cerebrospinal fluid (CSF) and plasma<sup>17</sup>. A mechanism for peripheral analgesic effect of oxytocin is inhibition of membrane depolarization of sensory neurons sensitive to Capsaicin and the increase in intracellular calcium<sup>18</sup>.

In this study, atosiban antagonized anti-nociceptive effect of oxytocin in both the tail flick and hot plate test and this indicate oxytocin via its receptors in spinal cord dorsal horn causing anti-nociceptive effect. In a study it was reported that oxytocin reduces the pain by effect on its receptor in dorsal horn of the spinal cord<sup>19</sup>. The results showed that in tail flick test oxytocin caused increase in latency and atosiban antagonized this effect that was expectable because these effects were shown in previous study<sup>20</sup>. It was also reported that intrathecal injection of atosiban has a hyperalgesic effect in rats with inflammation. On the other hand, an intrathecal injection of atosiban alone has no effect on the mechanical and thermal stimulation in normal rats<sup>17</sup>.

Oxytocin effected on the recipient of the Mu ( $\mu$ ) and Kappa ( $\kappa$ ) opioid and caused the analgesia<sup>21</sup>. Also, some of studies suggested that the analgesic effects of oxytocin, at least in part of its effects induced by the Delta ( $\delta$ ) and Kappa ( $\kappa$ ) opioid receptors<sup>22</sup>. In our study, Oxytocin increase morphine analgesia and atosiban blocked this effect of oxytocin. However atosiban did not affect morphine analgesia alone. This shows that oxytocin receptors did not play role in morphine analgesia as previous study.

Oxytocin receptor antagonist atosiban blocked oxytocin anti-nociceptive effect via oxytocin receptors and decreased morphine analgesic effect increased by oxytocin without oxytocin receptors. In addition, Atosiban showed hiperalgesic effect by oxytocin receptor but it did not effect morphine analgesic effect alone. Briefly, oxytocin reseptors may play role in regulating nociception directly but it may not play role in morphine analgesia directly.

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