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Original Research Article

Role of dopaminergic system in oxytocin analgesia: The missing part in a puzzle

Yaşar Taştemur[1], Ahmet Şevki Taşkıran[2]*, Ahmet Altun[3], Ahmet Kemal Filiz[2], Kader Gülmez[2], Kaan Çimen[1], Ercan Özdemir[2]
[1]Department of Anatomy, [2]Department of Physiology, [3]Department of Pharmacology, Cumhuriyet University Medical Faculty, Sivas, Turkey

For correspondence:* ***Email:* *ahmettaskiran@cumhuriyet.edu.tr;* ***Tel:*** *+90 346 219 1010;* ***Fax:*** *+90 346 219 1602*

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Abstract

Purpose: *To investigate the analgesic effects of oxytocin (OT) and elucidate the role of dopaminergic system in its mechanisms.*
Methods: *In this study, 72 male (n=6 for each group) 230-250 gr Wistar Albino rats were used. Firstly, dose studies were performed with 100 µg/kg, 200 µg/kg and 400 µg/kg to determine the optimal analgesic effect of oxytocin. Optimal dose was found at 200 µg/kg, and then animals were divided into nine groups: Saline, D1 agonist (SKF 38393; 0.1 mg/kg), D1 antagonist (SCH-23390; 0.1 mg/kg), D1 agonist + oxytocin, D1 antagonist + oxytocin, D2 agonist (Cabergoline; 0,5 mg/kg), D2 antagonist (Sulpride; 10 mg/kg), D2 agonist + oxytocin and D2 antagonist + oxytocin. Serum physiologic saline was given to the saline group and other drugs were administered intraperitoneally at the indicated doses. Tail-flick and hot-plate tests were used to measure analgesic effects. Analgesic tests were measured in 30 min-intervals (at 30th, 60th, 90th, and 120th min) and recorded in seconds. To evaluate maximum antinociceptive effect (% MPE), the tail-flick and hot-plate latencies were converted to the anti-nociceptive effectiveness*
Results: *The results show that D1 antagonist SCH-23390 (0.1 mg/kg) and D2 agonist cabergoline (0.5 mg/kg) created strong analgesia while the D1 agonist SKF 38393 (0.1 mg/kg) and D2 antagonist sulpiride (10 mg/kg) did not have any analgesic effect. However, only D2 antagonist sulpiride blocked the analgesic effect produced by OT*
Conclusion: *OT may be one of the primary agents participating in spinal analgesia, and the dopaminergic system is one of the central mechanisms of action for this important molecule. The dopaminergic system may also be one of the targets for 'descending' analgesic system.*

Keywords: *Oxytocin, Tail flick, Hot plate, Dopaminergic, Analgesic, Antagonist, Agonist*

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INTRODUCTION

Oxytocin (OT) is an amine peptide synthesized in the paraventricular (PVN) and the supraoptic (SO) nuclei of the hypothalamus. OT is released from the posterior pituitary into the systemic circulation, where it plays a fundamental role in mammalian labor and lactation through its peripheral actions [1], and modulates numerous functions including maternal bonding and social

affiliation through its central actions [2,3]. Recently, the effect of OT on pain sensitivity in both humans and animals has been increasingly investigated by several research groups, including ours.

The first important mechanism linking OT and pain involves hypothalamospinal oxytocinergic pathways. Stimulation of the PVN or administration of OT activates presynaptic OT receptors (OTRs) located superficially in the dorsal horn (Laminae I and II), which subsequently excites inhibitory GABAergic interneurons. Activation of GABAergic interneurons, in turn, pre-synaptically inhibits A Delta fiber and C fiber signals at nociceptive-specific and wide-dynamic-range neurons in Laminae I and II [4–6]. These effects can be reversed by selective OTR antagonists (d(CH2)5[Tyr(Me), Thr, Tyr-NH29]OVT) [7].

The second potential mechanism linking OT and pain involves an indirect pathway through the endogenous opioid system. Injection of opioid receptor (OR) antagonists has been reported to partially block the analgesic effects of OT. Regarding the relationship between OT and endogenous opiates, at least two potential mechanisms exist: OT can stimulate the release of endogenous opioids in the brain [8,9], and OT can bind to ORs and act as an orthosteric agonist or an allosteric modulator.

Dopamine (DA) is a catecholamine neuro-transmitter best known for its role in movement, cognition, and reward. However, the role of DA in nociception has been addressed in different basic and clinical studies [10]. Dopaminergic innervations inside the CNS arise from substantia nigra (SN), ventral tegmental area (VTA), and hypothalamus and reach numerous supraspinal structures and also areas in the SC [10,11]. In the basal ganglia, stimulation of mesolimbic dopaminergic neurons in the nucleus accumbens (NAc) and ventral striatum, either pharmacologically or provoked by stress conditions, has shown an analgesic effect. This effect appears to be mediated by D2 receptors as D2 agonist strengthens it and is blocked by a specific D2 antagonist eticlopride; D1 receptors did not show a related activity [12].

Additionally, microinjection of D2 agonist (quinpirole) in NAc inhibits the persistent phase of formalin-induced nociception in a dose-dependent manner [13]. Stimulation of VTA/SN has demonstrated an analgesic effect against chronic pain [14]. In Parkinson disease (PD), where there is a disruption in DA transmission, clinical evidence shows a close relation between pain-related symptoms and DA depletion [15]. Pain thresholds (heat and cold thresholds) are reduced in PD patients; this effect is reversed by L-DOPA administration [16].

Although the participation of the dopaminergic system in nociception is well documented over the years, there is however only one study that tried to a build a hypothesis to explain the involvement of the dopaminergic system in OT analgesia.

In the present study, we aimed to investigate the relationship between OT analgesia and the dopaminergic system, and provide more details on the mechanism of action of OT.

EXPERIMENTAL

Animals

The experiments were performed using adult male Wistar rats weighing 230 - 250 g (n = 72). The animals were fed a standard laboratory diet and water *ad libitum,* kept at 22 ± 2 °C with a 12-h light/dark cycle in a closed room, which had a lighting system controlled by timers. Animals were acclimatized to laboratory conditions before the test. All experiments were carried out blindly between 09:00 and 17:00 h (n = 6 in each experimental group in the study). The Cumhuriyet University Animal Ethics Committee approved the experimental protocols (approval no. 65202830-050.04.04).

Experimental protocol

At the beginning of the study, the anti-nociceptive effect of 100, 200 and 400 µg/kg in 1 ml/kg serum physiologic OT was given intraperitoneally (i.p.) to determine the optimal dose of the OT for further experimentation. Optimal dose was found at 200 µg/kg and then animals were divided into nine groups: saline, D1 agonist (SKF 38393; 0.1 mg/kg), D1 antagonist (SCH-23390; 0.1 mg/kg), D1 agonist + oxytocin, D1 antagonist + oxytocin, D2 agonist (Cabergoline; 0,5 mg/kg), D2 antagonist (Sulpride; 10 mg/kg), D2 agonist + oxytocin and D2 antagonist + oxytocin. Serum physiologic was given to the saline group i.p.; other drugs were dissolved in serum physiologic and were administered i.p. at the indicated doses. Tail-flick and hot-plate tests were used to measure analgesic effects. Analgesic tests were measured at 30 min-intervals (at 30th, 60th, 90th, and 120th minutes) and recorded in seconds. In order to evaluate the percentages of maximum antinociceptive effect (% MPE), the tail-flick and hot-plate latencies were converted to % MPE.

Tail-flick test

We used a standardized tail flick apparatus (May TF 0703 Tail-flick Unit, Commat, Turkey) to evaluate thermal nociception. The radiant heat source was focused on the distal portion of the tail at 3 cm after administration of the vehicle or study drugs intraperitoneally. Following vehicle or compound administration, tail-flick latencies (TFL) were obtained. The infrared intensity was adjusted so that basal TFL occurred at 2.8 ± 0.4 second. Animals with a baseline TFL below 2.4 or above 3.2 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 the tail-flick test is generally attributed to central mechanisms [21,22].

Hot-plate test

The antinociceptive response on the hot-plate is considered to result from a combination of central and peripheral mechanisms [22]. In this test, animals were individually placed on a hot-plate (Eddy's Hot-Plate) with the temperature adjusted to 55 ± 1°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 to avoid damage to paws.

Data analysis

The maximum possible effect (% MPE) was calculated for each rat at each dose and time point according to Eq 1.

MPE (%) = {(P – B/C – B)}100 …… (1)

where P, B and C are post-latency, baseline latency and cutoff latency, respectively.

Statistical analysis

The results obtained are expressed as mean ± SEM (standard error of the mean). The effect of anti-nociception was measured, and the mean of % MPEs in all groups was calculated. The data was analyzed by analysis of variance followed by the Tukey test. $P < 0.05$ was considered significant.

RESULTS

Analgesic effect of oxytocin (dose trial)

Even though the analgesic effect of oxytocin has been already successfully shown in literature, we needed to conduct a dosing trial to decide which doses of oxytocin we have to use for experimental setup. After preliminary studies, we narrowed the range between 100 and 400 µg/kg. Oxytocin created time and dose-dependent analgesic effects for all doses, both in the tail flick and hot plate tests. There was a significant difference between 100 and 200 µg/kg regarding the analgesic effect ($p < 0.05$). The dose of 200 µg/kg created a significantly higher analgesic effect when compared to 100 µg/kg. Nevertheless, there was no significant difference between the 200 and 400 µg/kg dose ($p < 0.05$). Hence 200 µg/kg was chosen for the rest of the experiments as the submaximal dose to be used (Figure 1 A and B).

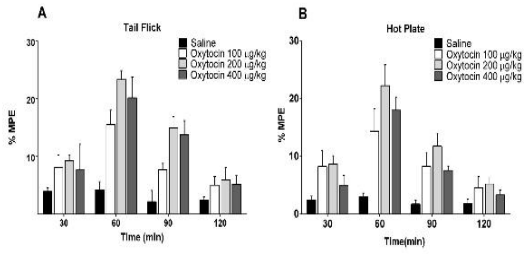

Figure 1: A) Tail flick latencies of 100, 200, and 400 µg/kg oxytocin; B) hot plate latencies of 100, 200, and 400 µg/kg oxytocin

Role of D1 receptors in analgesic effect of oxytocin

The analgesic effect of oxytocin has been re-evaluated in the presence of D1 and D2 receptor agonist and antagonist to elucidate the role of the dopaminergic system in the analgesic effect of oxytocin.

Administration of D1 agonist alone did not create any algesic and analgesic effects. Furthermore, it did not have any effect on the analgesic effect of oxytocin. Both the tail flick and hot plate latencies were unchanged (same level) following the administration of oxytocin alone. On the contrary, administration of D1 agonist and D1 antagonist created a strong, time-dependent analgesic effect. However, the D1 antagonist had no effect on the analgesic effect of oxytocin (Figure 2 A and B, Figure 3 A and B).

Role of D2 receptors in Analgesic effect of Oxytocin

Administration of D2 agonist alone created a time-dependent analgesic effect similar to the D1 antagonist. On the other hand, the analgesic effect of oxytocin did not change in the presence of D2 agonist, and while D2 antagonist alone did not have any algesic or analgesic effect, it did

however reduce the analgesic effect of oxytocin significantly ($p < 0.05$).

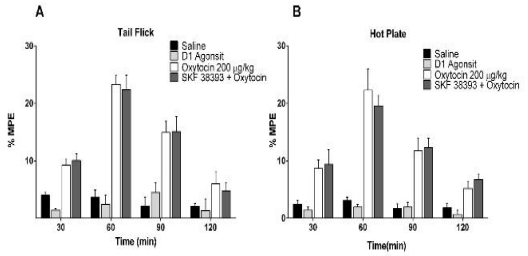

Figure 2: A) Tail Flick Latencies of 200 µg/kg Oxytocin in the Presence of D1 Agonist, B) Hot Plate Latencies of 200 µg/kg Oxytocin in the Presence of D1 Agonist

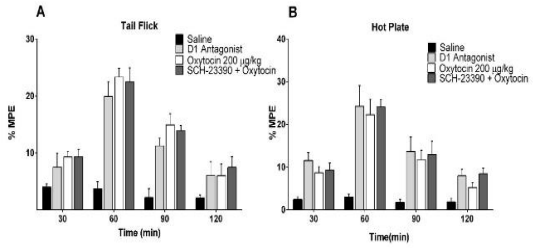

Figure 3: A) Tail Flick Latencies of 200 µg/kg oxytocin in the presence of D1 antagonist, B) hot plate latencies of 200 µg/kg Oxytocin in the presence of D1 antagonist

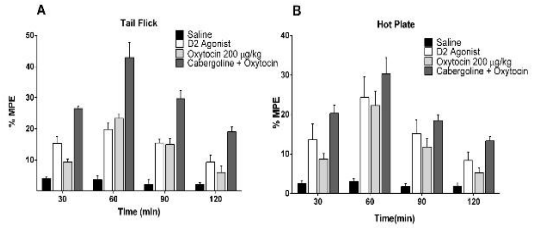

Figure 4: A) Tail Flick Latencies of 200 µg/kg Oxytocin in the Presence of D2 Agonist cabergoline, B) Hot Plate Latencies of 200 µg/kg Oxytocin in the Presence of D2 Agonist cabergoline

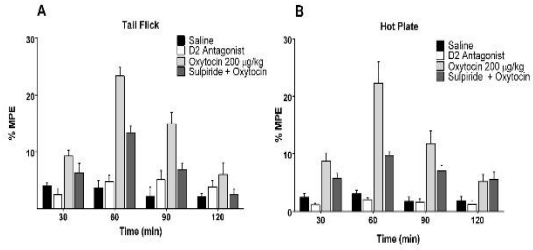

Figure 5: A) Tail Flick Latencies of 200 µg/kg Oxytocin in the Presence of D2 Antagonist, B) Hot Plate Latencies of 200 µg/kg Oxytocin in the Presence of D2 Antagonist

DISCUSSION

Oxytocin is a peptide produced in the paraventricular nucleus of the hypothalamus and associated with many important biological functions [17]. Recent studies have demonstrated the involvement of oxytocin in the modulation of nociception [18]. The emerging roles of oxytocin as an endogenous and exogenous analgesic have been reported in several clinical reports and basic researches using animal models [19]. In our study, consistent with literature [4,6,8], we demonstrated the analgesic effects of oxytocin in acute pain models. We also showed the optimal dose of oxytocin, which was also similar to the doses used in the studies in literature. Although all literature [4,6,8] is in agreement on the nociceptive effect of oxytocin, the mechanism for this action is still unclear. Currently, it is known that OT is able to reduce pain transmission by activation of several pathways. At spinal levels, OT enhances a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (GABA) release from spinal interneurons [20] located in the substantial gelatinousa [21].

Furthermore, OT reduces the GABA-evoked Ca^{2+} transients in trigeminal nociceptive neurons [22]. It is well known that oxytocin receptors (OTRs) and vasopressin 1a receptors (V1aRs) are structurally very similar receptors, and at certain locations, one agonist for each of them can easily affect the other and create a biological signal. However in small- and medium-sized dorsal root ganglion (DRG) neurons, the OT-induced inhibition of intracellular Ca^{2+} currents can be prevented by OTR blockers, not V1aR blockers [23]. This finding shows that V1aRs is one of the pathways for oxytocin to show its effects, but not the only one. Oxytocin also reinforces the GABAergic transmission (inhibition) in substancia gelatinosa via OTRs [21].

Qiu *et al* [24] suggest that peripheral OT can inhibit pain transmission at the periphery by activation of V1aR which in turn shrink the activity of acid-sensing ion channels (ASIC). There are also studies mentioning the relationship between oxytocin analgesia and opioid system and mü receptors [25]. It is obvious that we have made progress in understanding the underlying mechanisms of oxytocin analgesia. Nevertheless, it is also clear that there is a missing part to the puzzle. In the present study, the role of the dopaminergic system in oxytocin analgesia has been investigated as that missing part in the puzzle.

Dopamine has a role in both central and peripheral nociception. However, since exogenous oxytocin cannot pass the blood-brain barrier, the findings we had in our experimental set-up most likely were related to the peripheral nociception mechanism, most likely the spinal level. Dopamine-containing fibers and terminals are widely distributed in the spinal dorsal horn [26], which contains populations of both D1- and D2-class receptors [27]. It is shown that D1 receptor agonist did not have an affect, while D1 receptor antagonism induced the analgesic effect in an inflammatory pain model [28]. This is the same as our findings. In both tail flick and hot plate tests, we found that while D1 agonist did not affect algesia, D1 antagonist created a strong and time-dependent analgesic effect.

Furthermore, we showed that both D1 agonist and antagonist has no contribution in the analgesic effect of oxytocin, which indicates that D1 receptors do not take part in the mechanism of oxytocin-induced nociceptive effect. Liu et al [29] showed that Dopamine receptor D2, but not D1, mediates the descending dopaminergic pathway. This may be the reason D1 receptors are not participating in the analgesic effect of oxytocin. Hence since D1 receptors are coupled with the Gs/olf protein subunit of G-protein coupled receptors, inhibition of such a stimulating receptor would create inhibition in signaling, which in turn results in nociception. On the other hand, D2 receptors are coupled with the Gi/o protein subunit of G-protein coupled receptors [30].

Activation of spinal dopamine D2 receptors reduces pain-related behavioır following the establishment of inflammatory pain in both the affected and contralateral limb, while D2 receptor antagonism decreases pain thresholds [31]. Since D2 receptors are related to the descending nociceptive system, and they have an inhibitory nature. It should be expected that D2 agonists have an analgesic effect and D2 antagonists may reduce the analgesic effect created via D2 receptors. It was the case in our experimental design. We have shown that D2 agonist alone has a strong and time-dependent analgesic effect and the analgesic effect of oxytocin is prevented in the presence of D2 antagonist, which clearly indicates that oxytocin shows its analgesic effects at spinal level via D2 receptors. There may be more than one mechanism.

CONCLUSION

The dopaminergic system may have a more important role in the descending nociceptive pathways than it is thought. D2 receptors may mediate analgesic effects of many other molecules similar to the GABAergic system. To elucidate the mechanism of the analgesic effect of oxytocin and the role of the dopaminergic system at both spinal and central level, there is a need for further and advanced studies.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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