Acta Poloniae Pharmaceutica – Drug Research, Vol. 75 No. 1 pp. 205–210, 2018
ISSN 0001-6837
Polish Pharmaceutical Society

EFFECTS OF SINGLE INJECTION OF NALOXONE AND DAMGO WITHIN NUCLEUS ACCUMBENS SEPTI IN THE PLUS MAZE TEST IN RATS

CRISTIAN MORSUCCI[1], AINURA OKASOVA[1], DANIELA MULET[1], GRACIANA GALIANA[1], VANESA RODRÍGUEZ[1], GUSTAVO C. BAIARDI[2], JOSÉ VICENTE LAFUENTE[3], PABLO ADOLFO ELÍAS[1], ADRIANA I. LANDA[1], MARTA SOAJE[3] and PASCUAL A. GARGIULO[1]

[1]Laboratory of Neurosciences and Experimental Psychology, Department of Pathology, Faculty of Medical Sciences, National University of Cuyo, 5500 Mendoza, Argentina. ONICET
[2]Laboratory of Neuropharmacology, Institute of Biological and Technological Research (IIBYT-CONICET), National University of Córdoba, Faculty of Chemical Sciences, Catholic University of Córdoba, 5017 Córdoba, Argentina
[3]Laboratory of Clinical and Experimental Neurosciences (LaNCE). Faculty of Medicine and Odontology, University of the Basque Country, Spain

Abstract: Nucleus accumbens septi (NAS) is studied because its relations with cognition and anxiety. Its pharmacological manipulation is widely used in experimental psychopathology to reproduce psychotic signs and symptoms in animal models. In the present study, the effect of the injection of an agonist and a μ-receptor antagonist in this structure is assessed. Holtzman strain male rats (240-290 g) were cannulated bilaterally in NAS. One week after the injection they were subjected to an anxiety test, prior saline injection (controls), DAMGO ([D-Ala2, N-MePhe4, Gly-ol]-encephalin, opioid agonist) or naloxone (opioid antagonist). We evaluated the set of parameters classically considered in our laboratory (open arm time, time per entry, open arm entries, closed arm entries, open/closed arm quotient, open and closed arm ends arrivals, rearing, fecal bowls and grooming behaviors. There was only a significant increase in the length of stay in the open arm with the injection of DAMGO (0.2 μg/1 μL, $p < 0.05$) and a significant increase in grooming behaviors with naloxone (1 μg/1 μL, $p < 0.001$), compared with saline controls (1 μL). We conclude that the receptor stimulation in NAS generates effects compatible with anxiolysis, and blocking of such receptor in said structure results in an increase in grooming behaviors.

Keywords: opioids, opiates, nucleus accumbens septi (NAS), anxiety, grooming, perception, plus maze test

The nucleus accumbens septi (NAS) of the forebrain has been studied by our group starting from clinical evidences regarding perceptual dysfunctions in schizophrenia (1-3). These clinical and experimental conjunct of findings led to a reinterpretation of the pathophysiology of schizophrenia and its nuclear symptoms (4-10). There are convincing evidences regarding the role of NAS in cognitive phenomena, such as acquisition in the process of perception and learning (11-15), and they have been related to the pathophysiology of schizophrenia (6, 9, 10).

The presence of opioid receptors in the NAS has been observed to regulate dopaminergic activity. Intracerebroventricular administration of specific opioid receptor agonists has shown that activation of the opioid μ receptor located in the same NAS or in the ventral tegmental area (16, 17) increases the extracellular concentration of dopamine in the NAS, measured by microdialysis. Given the role of NAS in anxiety (13), the present experiment sought to study the role of opioidergic transmission within the NAS measuring its behavioral consequences.

SUBJECTS, MATHERIAL AND METHODS

Subjects

In present experiment, 90-day Holtzman strain rats weighing 240-270 g, maintained under controlled conditions of temperature (22-24°C) and light (lights between 5:00 p.m. to 7:00 p.m.) were used. Food for rats and water were available *ad libitum*.

* Corresponding author: e-mail: gargiulo@lab.cricyt.edu.ar

Surgery

The animals were anesthetized with ether and stereotactically implanted with stainless steel cannulae bilaterally in NAS. The cannulas (15 mm) have a removable stylet to prevent clogging. After surgery, the animals were housed in individual boxes and maintained without disturbances for a recovery period of one week.

Drugs

DAMGO (opioid agonist, Handa et al., 1981; 0.2 mg/1 mL) and naloxone (opioid antagonist, 1 mg/1 mL) injected into the NAS dissolved in saline were used. The control group was injected with saline (1 mL).

Plus maze test

Apparatus

The plus-maze is a wooden apparatus formed by four arms, two open and two closed, opposite each other. The four arms are 50 cm long and 10 cm wide. The appliance is raised 50 cm from the floor. The closed arms have walls of 50 cm.

Test

The animals were injected 15 min before starting. After this period, the test starts and the duration of the test was 5 min. During the test the following parameters were considered: time in the open arm, total number of entrances to both arms, entrances to the open arm, entrances to the closed arm, preference quotient (entries in the open arm/entries in the closed arm, open/closed arm quotient), the time/entrance ratio in the open arm, aerial exploration, arrivals at the end of the open arm, grooming and fecal boli.

Histology

After test, rats were sacrificed with an ether excess. Brains were removed and fixed in formalde-

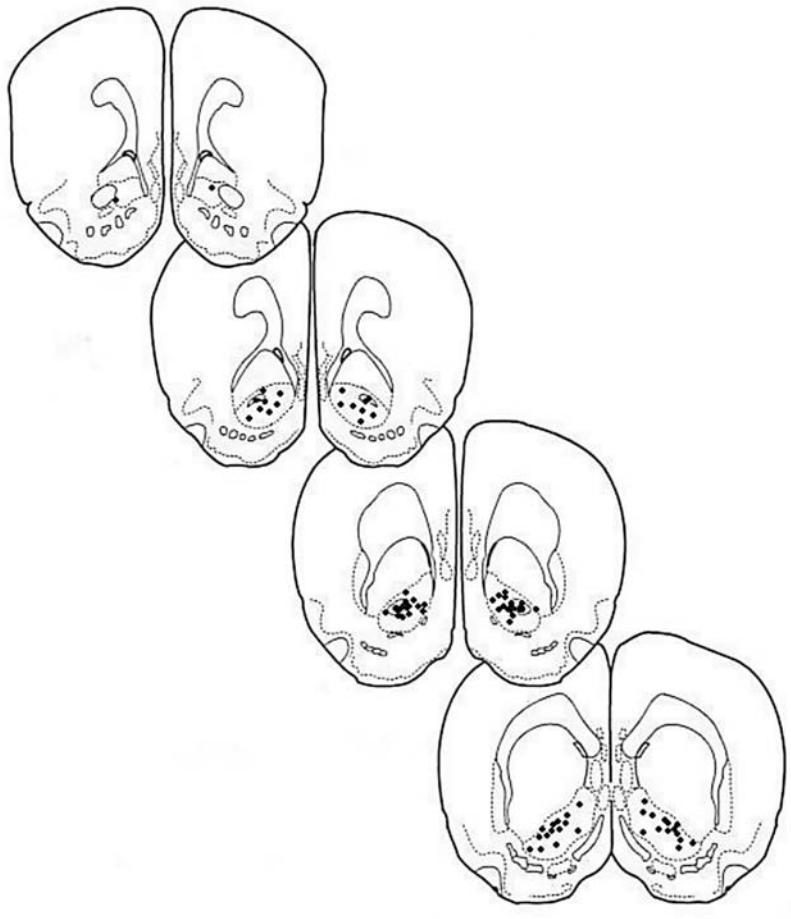

Figure 1. Location of the injection site is shown in frontal brain sections. All animals considered had correct cannula placements. ●: Cannulae placements (Pellegrino et al. 1979)

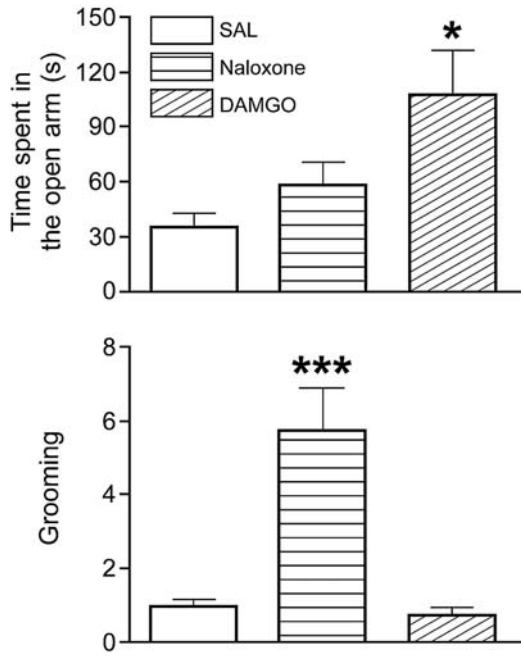

Figure 2. Top: Exploration time in open arm after saline (SAL the injection (1 µL, n = 17), naloxone (1 µg/1 mL, n = 23) and DAMGO (0.2 µg/1 µL, n = 20). Bottom: Grooming behavior after saline injection (1 µL, n = 17), naloxone (1 µg/1 µL, n = 23) and DAMGO (0.2 µg/1 µL, n = 20). The results are shown as the mean ± standard error of the mean (SEM, * = $p < 0.05$, *** = $p < 0.01$)

hyde solution (20%). Five days later, brains and position of the cannula were observed (18). Location of the injection site was observed and translated to frontal brain section graphics. All animals considered had correct cannula placements.

Data analysis

Significance was studied using the ANOVA test followed by the Student-Newman-Keuls test. In all cases a value of $p < 0.05$ (two tails) was considered significant. Results are reported as means and standard error of the mean (SEM).

Bioethical Considerations

Bioethical and legal dispositions were considered in present experiments. Project approval criteria of the National University of Cuyo were followed, and legal aspects fulfilled. In all cases, guidelines set established by European Community Council (Directive 86/609/EEC) were respected.

RESULTS

ANOVA showed significant differences between the groups in the time spent in the open arm ($F\ 2.57 = 4.42$, $p < 0.05$). A significant increase in the time spent in the open arm was observed with the injection of DAMGO (0.2 µg/1 µL, $p < 0.05$ *vs.* saline and naloxone). In the grooming behaviors, significant differences were observed between the groups in the time of permanence in the open arm ($F\ 2.56 = 13.54$, $p < .0.0001$), with a significant increase in grooming behaviors with naloxone (1 µg/1 µL, $p < 0.001$ *vs.* saline and DAMGO).

DISCUSSION

Injection of drugs related to opioidergic transmission in NAS produces behavioral effects in the plus-maze test. The time spent in the open arm was increased by DAMGO injection (Fig. 2, top). This could be indicating that the stimulation of the µ opioidergic receptors would mediate anxiolytic effects. On the other hand, the antagonism of the same ones using naloxone induces an increase of the grooming behaviors (Fig. 2, bottom). The fact that the other parameters have not been modified by the treatment (see Table 1) can be considered a sign of the reliability of the method used. However, an interaction between opioidergic receptors and other types can

not be excluded. In this sense, as previously mentioned, opioidergic receptor agonists increase the concentration of dopamine (DA) in NAS, either by action in this structure or by action on ventral tegmental area (16, 17).

Something similar has been suggested for other opioidergic receptors. Thus, the dopamine-releasing action observed with fentanyl in NAS was induced both by systemic administration and by selective injection in ventral tegmental area or NAS. In the cited study, various manipulations have been made to act on the effect induced by fentanyl by injection into the NAS. This was abolished by the systemic administration of naloxone. It was also abolished by administration into the NAS of a D-Phe-Cys-Tyr-D-Trp-Om-Thr-Phe-Thr-NH_2 antagonist in a dose-dependent manner. Administration of a non-selective δ-opioid receptor antagonist, natrindol, also abolished the effect of fentanyl in a dose-dependent manner. Differential effects were observed when selective δ receptor antagonists were used. When blocking the effect of fentanyl on NAS with naltriben, a δ_2-opioid receptor antagonist, blockade was observed. This did not occur using (E)-7-benzylidenenaltrexone, a δ_1-opioid receptor antagonist. All of this has led to the suggestion that there is a close interaction between opioid receptor subtypes to modulate DA levels within the NAS. Again, the NAS here shows its relevance in anxiety processing, as previously described by us (13, 19). In this sense, the present study has a character of confirmation. The behaviors deployed in this experiment merit differential attention.

Grooming behaviors have been the subject of study by our team. Several endogenous ligands may increase it (20, 21). Some of them, such as corticotropin releasing hormone (CRH) may increase it, and may, in turn, induce an anxiogenic type frame (20, 21). However, what we observe in this study is an increase of the grooming behaviors with a decrease of the anxiety, evidenced in the increase of the time spent in the open arm. This leads to an alternative hypothesis for the explanation of this behavior.

One possibility is given by a possible increase in cutaneous sensitivity due to changes in sensory processing induced by the application of naloxone. The action of opiates has been classically related to the management of pain sensitivity. However, the present findings may be indicating a modulatory action of tactile epicritic sensitivity. This may well be exercised on the NAS, given the concentration of opiate receptors present in it, and the fact that this structure has been involved in sensory processing. In fact, a corticosteroid system has been proposed in pain processing (22). This system, consisting of the ventromedial prefrontal cortex and the NAS, has been linked to chronic pain but also to another sensory modality, such as tinnitus (22). This would denote a central action on the processing of non-painful sensory information. Thus, frontal cortex and NAS function have been proposed as part of a central gatekeeping system, which would filter more than one sensory modality by assessing the affective value and relevance of the stimuli (22).

The NAS also seems to process other sensory modalities. Thus, the interference of their function has been shown to interfere with the formation and consolidation of the taste memory (23, 24). This interference has been performed on both the muscarinic and glutamate receptor systems (23) and by a protein synthesis inhibitor applied to the NAS shell (24). Finally, it has been reported that the tactile skin stimulation produces an increase in DA release in the

Table 1. Values of open arm entries, closed arm entries, open/closed arm quotient, time per entry, extreme open arm arrivals, extreme closed arm arrivals, rearing and fecal boli. Rat groups: saline control (1 µL, n = 17), Naloxone (1 µg/1 µL, n = 23) and DAMGO (0.2 µg/1 µL, n = 20). Data are presented as the mean ± SEM.

	Sal n = 17	Naloxone n = 23	DAMGO n = 20
Open arm entries	2.12 ± 0.33	2.61 ± 0.27	2.85 ± 0.49
Closed arm eEntries	5.35 ± 0.60	6.00 ± 0.71	5.50 ± 1.13
Open / Closed arm quotient	0.49 ± 0.07	0.58 ± 0.10	1.22 ± 0.39
Time per entry	16.56 ± 2.63	29.62 ± 12.36	64.84 ± 23.26
Extreme open arm arrivals	1.53 ± 0.32	1.74 ± 0.35	1.45 ± 0.41
Extreme closed arm arrivals	5.76 ± 0.57	5.78 ± 0.74	5.20 ± 1.12
Rearing	8.47 ± 1.04	5.65 ± 1.13	6.05 ± 1.16
Fecal boli	1.23 ± 0.53	1.04 ± 0.34	0.95 ± 0.28

NAS (25). This latter finding would explain the function of the NAS system in the reaction, linking the tactile processing to this structure and giving reason to the groming behaviors, possibly secondary to an increase in the corporal tactile sensibility.

Opioids have been implicated in the pathophysiology of schizophrenia (26). In particular, De Wied's work, with a passive avoidance test, was pioneering and relevant at this point (27). In the present study, opiates were injected directly into the NAS, a structure traditionally linked to schizophrenia (see 6, 9, 10). The purpose was to study its effects in an anxiety test, continuing findings from other groups that cite our work (28). It is possible that new therapeutic strategies may become of the present findings. In fact, opioid peptides have also been implicated in possible therapeutic uses in schizophrenic psychoses (29).

We may conclude that μ receptor stimulation in NAS generates effects compatible with anxiolysis, and blocking of such receptor in said structure results in an increase in grooming behaviors. The role of opioid receptors in the NAS, and their behavioral relevance, becomes evident and clinical projections may be relevant.

Acknowledgments

We thank Daniel Dueńas for his invaluable cooperation with the graphics, and Mrs. Miriam Fraile for her technical contribution. The research reported is part of a collaborative project with Prof. J.D. Delius, Allgemeine Psychologie, Universität Konstanz, Germany supported by the Volkswagen Foundation (Grant Gargiulo-Delius: "Nucleus Accumbens Septi und kognitives Verhalten"). It was also supported by the First Neuroscience Grant of Latin American Technological Corporation Foundation (Fucotel), and the National University of Cuyo (Project 06/J438, approved by the University Council 4540-R/2013). We thank Mr. Marcos Constantino Josué Gargiulo for his invaluable help in drafting the manuscript. We thank also Mrs. Sara Roitman for her invaluable cooperation.

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Received: 31. 12. 2016