

ORIGINAL ARTICLE

Medicine Science 2021;10(3):737-40

Evaluation of the effects of dexmedetomidine and midazolam on plasma and cerebrospinal fluid beta-endorphin levels in rats

ID Canan Kucuk¹, ID Nihal Kadiogullari²¹29 Mayıs Hospital, Department of Anesthesiology and Reanimation, Ankara, Turkey²Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Department of Anesthesiology and Reanimation, Ankara, Turkey

Received 12 March 2021; Accepted 21 April 2021

Available online 09.08.2021 with doi: 10.5455/medscience.2021.03.081

Copyright©Author(s) - Available online at www.medicinescience.org

Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.



Abstract

Endorphins are endogenous peptides that play a role in the regulation of pain, behavior, neuroendocrine and autonomic functions. In our study, we aimed to investigate the role of β endorphins in these effects of midazolam and dexmedetomidine. In this experimental study, 36 rats were divided into 6 groups. Group 1: (control) (n=6): An average of 100-150 μ l CSF samples were obtained after general anesthesia was achieved. An intracardiac 3-4ml blood sample was then taken. Group 2: (sham) (n=6): intraperitoneal 1 mL saline was administered. For all other groups after general anesthesia; Group 3: (midazolam-1) (n=6): intraperitoneal 4mg/kg midazolam was administered. Group 4: (midazolam-2) (n=6): 12mg/kg midazolam was administered intraperitoneally. Group 5: (Dexmedetomidine-1) (n=6): Dexmedetomidine 50 μ g/kg was administered intraperitoneally. Group 6: (Dexmedetomidine-2) (n=6): Dexmedetomidine 150 μ g/kg was administered intraperitoneally. CSF and plasma samples were taken with the same method. All samples were stored at -80 °C. No statistically significant difference was found between the levels of rat CSF β -endorphin. However, when the plasma β -endorphin levels of rats were evaluated, there was a statistically significant difference between the groups. No difference was found between the levels of plasma β -endorphin in the patients who received midazolam, while plasma β -endorphin levels of 50 μ g/kg with dexmedetomidine were found to be lower than the control group. However, the values of the 150 μ g/kg dexmedetomidine group did not support this result. Different findings from both this study and in vitro and human studies; the number of subjects, the use of different drug doses, the differences in measurement times, the absence of control groups may be due to methodological differences, and more research is needed.

Keywords: β -endorphin, midazolam, dexmedetomidine

Introduction

Endorphins are endogenous peptides that bind to opioid receptors with opioid-like activity. Research shows that these endogenous peptides play a role in the regulation of pain, behavior, neuroendocrine and autonomic functions. Endogenous opioids are found in all vertebrates and most invertebrates. When applied to the central nervous system (CNS) β endorphins, which have a strong analgesic effect, do not show this effect when administered intravenously [1].

Midazolam is a benzodiazepine derivative that acts as an anxiolytic, hypnotic, anticonvulsive, muscle relaxant, and amnesic, affecting gamma aminobenzoic acid (GABA-A) receptors. This drug, which is known to have no analgesic effect, shows an analgesic effect when administered intrathecally [2] and a hyperalgesic effect when administered intraperitoneally [3].

Dexmedetomidine is an α_2 receptor agonist with sedative, analgesic, and anesthetic properties that are becoming increasingly common today. The potent analgesic activity after intrathecal and epidural administration of α_2 adrenergic agonists has made this drug group more attractive [4].

There are many studies on β -endorphin peptides. However, studies on the release of these peptides are insufficient.

Several physiological and behavioral effects of β -endorphin are available [5]. The most important effect is the inhibition of unhappy warnings in species, including humans. Supraspinal or spinal administration of β -endorphin to CNS is based on analgesia in humans [1], and behavioral testing in animals [6]. The neuronal linkages of β -endorphin-induced antinociception are not yet known. The effect of β -endorphin directly inhibits nociceptive pathways in the thalamus and cortex. The indirect effects of β -endorphin are mediated by the modulation of descending pathways regulating nociception [7]. The supraspinal antinociceptive effects of β -endorphin play an important role in the β , δ , ϵ receptors [8].

*Corresponding Author: Canan Kucuk, 29 Mayıs Hospital, Department of Anesthesiology and Reanimation, Ankara, Turkey
E-mail: canankucuk@yahoo.com

Measurement of cerebrospinal fluid (CSF): It is generally independent of plasma levels [9]. The advantage of this method is that it can be applied to people and compared to animal studies. The disadvantage is not knowing where the oscillation is made. Measurement of β -endorphins in cerebrospinal fluid (CSF) is a valid method to understand the possible role of β -endorphin in the brain in nociception modulation.

Jeffcoat has shown β -endorphins in 1978 in human CSF [10]. The ideal radioimmunoassay (RIA) is the administration of the antiserum that only recognizes the specific antigen. However, both the β -endorphin precursors and their derivatives cross-react. Studies have shown that the C-terminal antiserum only affects β -endorphins β -endorphin immunoreactive components of the N-terminal antiserum. The use of C-terminal RIA for the measurement of β -endorphin in CSF was approved [11]. Passing three ml of CSF into a Sep-Pak C-18 cartridge eliminated the interaction of β -LPH [12]. The mean basal β -endorphins in the lumbar and ventricular human CSF was 12.1 ± 3 pmol / L. It has been shown that age and gender do not affect CSF β -endorphin levels in healthy individuals [11]. The use of C-terminal antiserum is sufficient for the determination of β -endorphin in the smallest amount of CSF [13]. But extraction provides higher specificity.

Although studies are showing the effects of midazolam on β endorphins, there is no study on dexmedetomidine. In this experimental study, we aimed to investigate the role of β endorphins in the analgesic effects of midazolam and dexmedetomidine.

Materials and Methods

The experimental protocol of this study was examined and approved by the Hacettepe University Faculty of Medicine (HÜTF) ethics committee (Registration No: 2004-48), (Approval Date: 2/9/2004). In this study, a total of 36 rats weighing between 250 and 400 grams of male Sprague-Dawley were used. During the experiment, rats were kept in cages with dark 10 hours darkness for 14 hours and fed with standard laboratory food.

Sodium pentobarbital (Nembutal, 70 mg/kg) was injected intraperitoneally [14], which did not increase plasma β endorphin values and did not take part in opioid receptor activation [14], to provide general anesthesia for rats which were fasted for an hour.

Cysterna Magna puncture was performed stereotactically in all groups. For stereotactic (USA David Kopf Stereotactic rat adapter model: 920-1220-1520-1720) and stereotax used in the experimental studies and applied operations.

The study was performed on 6 groups.

Group 1 (control) (n = 6): After general anesthesia was achieved, the rats in this group were shaved with inion to the atlas. Field cleaning was done with Batikon. A 1 cm incision was made between the inion and atlas between the head and the head of the rat. The skin was passed, the occipital muscle was bluntly dissected from the occipital bone, the posterior cervical muscles were dissected bluntly, and the atlantooccipital membrane was revealed (Figure 1). The insulin syringe needle was inserted into the stereotaxic frame with a 0.45x13mm, 2ml syringe. An average of 100-150 μ l CSF samples was taken. An intracardiac 3-4ml blood sample was

taken. The samples were taken to the laboratory with ice-cold polyethylene tubes. CSF samples were centrifuged at 1000 rpm for 5 minutes at 4 °C. Plasma samples were centrifuged at 1600 °C for 15 minutes at 4 °C with the addition of 0.6TIU / ml Aprotinin. All samples were stored at -80 °C.

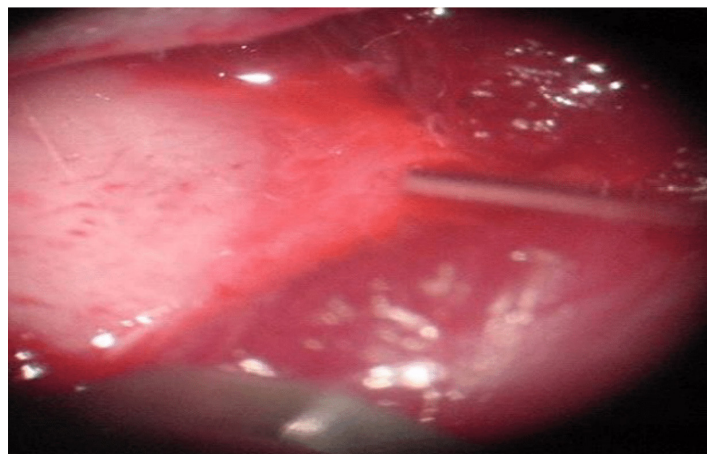


Figure 1. Stereotaxic application and introduction to atlanto-occipital membrane

Group 2: (sham) (n = 6): Under general anesthesia, rats in this group were administered intraperitoneal saline, and CSF and plasma samples were taken with the same method.

Group 3: (midazolam-1) (n = 6): Under general anesthesia, the rats in this group were given intraperitoneal 4 mg/kg midazolam, and then CSF and plasma samples were taken with the same method.

Group 4: (midazolam-2) (n = 6): After general anesthesia, 12 mg/kg midazolam was administered intraperitoneally to the rats in this group.

Group 5: (Dexmedetomidine-1) (n = 6): After general anesthesia, dexmedetomidine 50 μ g/kg was administered intraperitoneally to the rats in this group.

Group 6: (Dexmedetomidine-2) (n = 6): After general anesthesia, dexmedetomidine 150 μ g/kg was administered intraperitoneally to the rats in this group. Ic frame detection (USA David Kopf model: 1530 stereotaxic frame assembly) was used.

Sep-Column was used for plasma extraction in the HU Faculty of Pharmacy. After extraction, the samples were lyophilized. Because these procedures were not required for CSF, only plasma was performed. β -endorphins (Rat) Radioimmunoassay (RIA) kit (Phoenix Pharmaceuticals, Inc., Cat No: EK-022-33) was used to measure the RIA laboratory in the HUTF Nuclear Medicine Department.

Results

Both CSF and plasma β -endorphin levels of 6 groups were evaluated using the Kruskal-Wallis variance analysis test. Mann-Whitney-U test was used for bilateral evaluation. The procedures performed during the experiment were generally well tolerated by rats.

No statistically significant difference was found between the BOS β -endorphin levels of the 6 groups (Kruskal Wallis di2 = 3.156,

$p < 0.67$) (Table-1). However, a statistically significant difference was found between groups (Kruskal Wallis $\chi^2 = 15.67$, $p < 0.08$) (Table-1). To determine the differences in plasma β -endorphin levels, groups were evaluated with the control group. No difference was found between the levels of plasma β -endorphin in the patients who received midazolam, while plasma β -endorphin levels of 50 $\mu\text{g/kg}$ with dexmedetomidine were found to be lower than the control group. However, the values of the 150 $\mu\text{g/kg}$ dexmedetomidine group did not support this result ($U = 9$, $p < 0.15$).

Table 1. BOS and Plasma β -endorphin values

GROUPS	CSF (pg / L)	PLASMA (pg / L)
Group 1	0.65 (0.10-4.59)	2.38 (2.01-2.85)
Group 2	0.34 (0.10-6.95)	1.86 (0.19-6.40)
Group 3	0.57 (0.11-1.08)	1.52 (0.82-2.72)
Group 4	0.80 (0.13-2.56)	2.77 (0.77-3.27)
Group 5	1.00 (0.18 - 2.06)	1.06 (0.54-2.39)
Group 6	0.99 (0.60-1.34)	2.09 (1.25-2.53)
p	0.67	0.08
Values: median (min-max)		

Discussion

In the 6 groups compared in this study, no difference was found between the levels of rat CSF β -endorphin. Plasma β -endorphin levels were not significantly different in rats treated with midazolam, whereas plasma β -endorphin levels with 50 $\mu\text{g/kg}$ dexmedetomidine were found to be lower than the control group. However, since we could not achieve these results in the 150 $\mu\text{g/kg}$ dexmedetomidine group, we think that dexmedetomidine does not affect plasma β -endorphin levels at the doses administered.

Studies are showing that pentobarbital does not increase plasma β -endorphin levels or has no effect on opioid receptors [14]. Takashi and colleagues [15] in rats in their study 70mg/kg pentobarbital intraperitoneal administration of CSF and plasma β -endorphin levels were measured. In our study, CSF and plasma samples were obtained by providing general anesthesia to rats with the same dose of pentobarbital.

The dose of 4 mg/kg midazolam administered in our study was an anesthesia induction dose administered to rats [16] and the equivalent dexmedetomidine dose was calculated as 50 $\mu\text{g/kg}$ [17]. To reveal the acute effects of these drugs, doses that are 3 times more than those routinely used were used. These doses were well tolerated by rats.

Cysterna Magna cannulation or puncture was performed in many studies to determine β -endorphin levels. We preferred the puncture technique instead of cannulation in our study because it was shown that cysterna magna cannulation did not affect the β -endorphin levels in rats for the first hour and changed the levels of β -endorphin in the following hours [18]. In this study, we obtained 100-150 l CSF in 3-4 minutes with the cisterna magna puncture. The amount obtained was the whole of CSF found in rats and was the same as that obtained in other studies [15]. Because the volume required for measurement in the β -endorphin (rat) IUD kit was 100 l, measurements could be performed once.

Measurement of β -endorphin in cerebrospinal fluid is a valid method for understanding the effect of β -endorphin in the brain in nociception modulation. There are reports that plasma β -endorphins, which are known to have a short half-life, are independent of CSF β -endorphin levels and do not play a role in pain or nociception [19]. The endogenous opioid peptide β -endorphins, when administered to the central nervous system (supraspinal or spinal), have a strong analgesic effect in humans, and in animal behavioral assays antinociception [1].

In this study, we could not determine where the β -endorphin release was performed because we could not perform the tissue concentration measurement. In a study of mouse pituitary cells in vitro, propofol has been shown to inhibit β -endorphin secretion and in another study, ketamine has been shown to stimulate β -endorphin release [20, 21]. In a study that investigated the effects of morphine, midazolam, and both drugs on β -endorphin levels in rats in 11 groups for 4 days (saline-saline, midazolam-saline, saline-morphine, and midazolam-morphine) in the midazolam-saline group at the level of β -endorphin in the medulla while rising; hippocampus, striatum, adrenal glands were found to fall. While no change was observed in the saline-morphine group, β -endorphin levels in plasma were found to be low in the midazolam-saline group [22].

The antinociceptive effect of midazolam which is thought to have no analgesic effect when administered intrathecally to dogs is not antagonized by naloxone [23]. However, the spinal analgesic effects of midazolam, which are antinociceptive in the studies of Serrao et al. [23] have been antagonized by naloxone. In another study, midazolam was administered intraperitoneally to the rats and a hyperalgesic effect was observed [23]. The effect of midazolam administered to people with intrathecal route is effective in the treatment of chronic back pain [23]. In a study by Mizutani et al. [24] plasma β -endorphin levels were lower in patients treated with midazolam than the sevoflurane group.

In another study evaluated preoperatively and preoperatively after midazolam, sufentanil, isoflurane balancing anesthesia, no change in β -endorphin levels was observed in plasma [25].

Plasma β -endorphin levels increased after nitro oxide/oxygen/ sevoflurane compared to control concentrations, but no increase after midazolam/fentanyl/oxygen air anesthesia [26].

In a study by Crozier et al. [27] in midazolam, increased plasma β -endorphin levels were prevented. In our study, it was found that the two doses of midazolam did not affect the rat CSF and plasma β -endorphin levels.

The relationship between plasma β -endorphin levels and preoperative anxiety has not been established in patients undergoing oral diazepam and midazolam [28]. In another in vitro study, caffeine stimulated plasma β -endorphin levels, while the same effect could not be demonstrated in CSF [29].

In a study comparing halothane and alpha chlorolose in cats, halothane has been shown to increase β -endorphin levels and alpha chlorolose does not affect [30].

As a result; different findings from both this study and in vitro

and human studies; the number of subjects, the use of different drug doses, the differences in measurement times, the absence of control groups may be due to methodological changes, and more research is needed.

Limitations

Since our study is an animal experiment, the number of subjects used in each group is low. The results of the statistical tests give more meaningful results in the case of high sample numbers. Keeping the sample size of our study low due to compulsory reasons constitutes the most important limitation.

Conflict of interests

The authors declare that they have no competing interest.

Financial Disclosure

All authors declare no financial support.

Ethical approval

The experimental protocol of this study was examined and approved by Hacettepe University Faculty of Medicine (HÜTF) ethics committee (Registration No: 2004-48), (Approval Date: 2/9/2004).

References

- Oyama T, Jin T, Yamaya R et al. Profound analgesic effects of beta-endorphin in man. *Lancet*. 1980;1:122-4.
- Serrao JM, Stubbs SC, Goodchild CS et al. Intrathecal midazolam and fentanyl in the rat: evidence for different spinal antinociceptive effects. *Anesthesiology*. 1989;70:780-6.
- Niv D, Davidovich S, Geller E et al. Analgesic and hyperalgesic effects of midazolam: dependence on route of administration. *Anesth Analg*. 1988;67:1169-73.
- Kalso EA, Poyhia R, Rosenberg PH. Spinal antinociception by dexmedetomidine, a highly selective alpha 2-adrenergic agonist. *Pharmacol Toxicol*. 1991;68:140-3.
- De Wied D, Jolles J. Neuropeptides derived from pro-opiocortin: behavioral, physiological, and neurochemical effects. *Physiol Rev*. 1982;62:976-1059.
- Yaksh TL, Gross KE, Li CH. Studies on the intrathecal effect of beta-endorphin in primate. *Brain Res*. 1982;10;241:261-9.
- Jensen TS. Endogenous antinociceptive systems: studies on spinal and supraspinal modulating mechanisms with particular reference to monoaminergic and opioid systems. *Acta Neurol Scand Suppl*. 1986;108:1-35.
- Porreca F, Burks TF. Supraspinal opioid receptors in antinociception. In: A. Herz (eds.), *Handbook of experimental pharmacology*, Vol. 104/II, Opioids, Springer-Verlag, Berlin, 1993;21-51
- Fishman RA. *Cerebrospinal fluid in diseases of the nervous system*. 2nd ed., Philadelphia: W.B. Saunders Company, 1992.
- Jeffcoate WJ, Rees LH, McLoughlin L et al. beta-Endorphin in human cerebrospinal fluid. *Lancet*. 1978;15;2:119-21.
- Bach FW, Schmidt JF, Faber T. Radioimmunoassay of beta-endorphin in ventricular and lumbar cerebrospinal fluid. *Clin Chem*. 1992;38:847-52.
- Laatikainen TJ, Salminen KR, Sulkava RO, et al. Determination of beta-endorphin and beta-lipotropin in human cerebrospinal fluid. *Clin Chim Acta*. 1985;15;145:325-9.
- Smith R, Owens PC, Lovelock M, et al. Acute hemorrhagic stress in conscious sheep elevates immunoreactive beta-endorphin in plasma but not in cerebrospinal fluid. *Endocrinology*. 1986;118:2572-6.
- Ramirez-Gonzalez MD, Barna I, Wiegant VM, et al. Effect of anaesthetics on the release of beta-endorphin-immunoreactivity in rat plasma. *Life Sci*. 1991;48:1371-7.
- Yamamoto T, Sako N, Maeda S. Effects of taste stimulation on beta-endorphin levels in rat cerebrospinal fluid and plasma. *Physiol Behav*. 2000;69:345-50.
- Ozkose Z, Menten BB, Gorgul A. In: *Anesthesia in experimental animals*. 1998;122.
- Aantaa R, Jaakola ML, Kallio A, et al. A comparison of dexmedetomidine, and alpha 2-adrenoceptor agonist, and midazolam as i.m. premedication for minor gynaecological surgery. *Br J Anaesth*. 1991;67:402-9.
- Barna I, Sweep CG, Veldhuis HD et al. Differential effects of cisterna magna cannulation on beta-endorphin levels in rat plasma and cerebrospinal fluid. *Acta Endocrinol (Copenh)*. 1988;117:517-24.
- Houghten RA, Swann RW, Li CH. beta-Endorphin: stability, clearance behavior, and entry into the central nervous system after intravenous injection of the tritiated peptide in rats and rabbits. *Proc Natl Acad Sci U S A*. 1980;77:4588-91.
- YaDeau JT. Inhibition of regulated neuropeptide secretion from mouse pituitary cells by propofol. *Anesth Analg*. 1996;83:611-7.
- YaDeau JT, Morelli CM, Billingsley JK. Ketamine stimulates secretion of beta-endorphin from a mouse pituitary cell line. *Reg Anesth Pain Med*. 2003;28:12-6.
- Rattan AK, Tejwani GA. Effect of chronic treatment with morphine, midazolam, and both together on beta-endorphin levels in the rat. *Brain Res Bull*. 1996;41:335-41.
- Raj PP (ed): *Practical management of pain*. Lang E. Niv D: Management needs and ideas for better treatment, 3rd edition. Mosby, Inc.2000, p. 1005-6.
- Mizutani A, Hattori S, Yoshitake S et al. Effect of additional general anesthesia with propofol, midazolam or sevoflurane on stress hormone levels in hysterectomy patients, receiving epidural anesthesia. *Acta Anaesthesiol Belg*. 1998;49:133-9.
- Roth-Isigkeit AK, Schmucker P. Postoperative dissociation of blood levels of cortisol and adrenocorticotropin after coronary artery bypass grafting surgery. *Steroids*. 1997;62:695-9.
- Mizutani A, Taniguchi K, Miyakawa H et al. Stress hormone response during midazolam/fentanyl anesthesia combined with epidural anesthesia for abdominal total hysterectomy. *Masui*. 1996;45:276-80.
- Crozier TA, Beck D, Schlaeger M et al. Endocrinological changes following etomidate, midazolam, or methohexital for minor surgery. *Anesthesiology*. 1987;66:628-35.
- Pippingskold K, Lehtinen AM, Laatikainen T et al. The effect of orally administered diazepam and midazolam on plasma beta-endorphin, ACTH and preoperative anxiety. *Acta Anaesthesiol Scand*. 1991;35:175-80.
- Arnold MA, Carr DB, Togasaki DM et al. Caffeine stimulates beta-endorphin release in blood but not in cerebrospinal fluid. *Life Sci*. 1982;6;31:1017-24.
- Browning JL, Heizer ML, Widmayer MA et al. Effects of halothane, alpha-chloralose, and pCO₂ on injury volume and CSF beta-endorphin levels in focal cerebral ischemia. *Mol Chem Neuropathol*. 1997;31:29-42.