



Effect of propofol on endogenous morphine in serum

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ABSTRACT

Background & Objective: Studies have indicated that there is alteration of endogenous morphine after surgical interventions. As surgical interventions either clinical or experimental are carried out under anaesthesia. So, it cannot be concluded that the altered endogenous morphine is due to only surgical trauma. The research was, is there any anaesthetic effect on the endogenous morphine?

Objective of the current study was to determine any effect of propofol anaesthesia on the endogenous morphine.

Methodology: Young male rats were injected either propofol anaesthesia or intralipid control and scarified at day 1 and day 3 to obtain blood and serum samples. Endogenous morphine was quantified in the samples by using a morphine specific enzyme-linked immunosorbent assay kit. Results: one way ANVOA showed a significant effect of treatment at day 1 between propofol and intralipidic control ($p < 0.05$). There was also significant effect of propofol between days of treatment with propofol ($p < 0.05$) but not with intralipid controls ($p < 0.05$).

Conclusion: The current study showed that propofol anaesthesia increases the level of endogenous morphine at day 1 of the treatment. This increase of endogenous morphine till day 1 might show a protective effect as analgesic in reaction to propofol injection. However, this effect was diminished at day 3 of the treatment.

Key words: Endogenous morphine; Propofol; Anaesthesia; Circadian rhythms; Intralipid

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INTRODUCTION

It has been believed that the synthesis of morphine is only limited to plants. However, for the first time in 1985, it was evidenced that morphine is also found in animals and it was found in toad's skin.¹ Later on, endogenous morphine was isolated in many different mammalian tissues including brain, liver, skin, adrenal gland and body fluids as well.² Further, receptors for endogenous morphine were isolated and interestingly, these receptors were observed in immune cells including human granulocytes and agranulocytes.³ On the basis of such studies, it may be proposed that there might exist a relationship between endogenous morphine and inflammatory process and

the substances which are responsible in alteration of inflammatory processes. Studies have also proposed that endogenous morphine is responsible in down-regulation of immune response to inflammation and tissue injury.⁴

Postoperative state is the best condition to understand the process of inflammatory and immune response. Reviews on clinical and experimental studies have indicated that surgical procedures are associated with immune reactions in the form of innate and acquired immunity. It has also been evidenced that the severity of immune reaction is positively correlated with the extent of surgical procedure and other factors including type of procedure or treatment given etc.⁵ It has also been demonstrated

that endogenous morphine levels are increased after cardiac surgery and it is assumed that this increase may be due to the inflammatory reaction caused by the cardiac surgery.⁶ Inflammation and sepsis is also associated with surgical procedures and level of endogenous morphine has also been observed to be increased in serum of such patients.⁷ However, this increase in endogenous morphine may also be due to some other factors including any synergistic effect of anesthesia, circadian effect etc.

Usually, surgical procedures are associated with anesthesia and it cannot be assumed that only inflammatory reactions are responsible for increase of endogenous morphine but there may be a direct effect of anesthetics. So, we conducted the current study to investigate any anesthetic effect on the endogenous morphine.

METHODOLOGY

Animals; Young male Sprague dawley rats were housed for fifteen days to acclimatize in a temperature of 24 ± 1 °C and humidity ($60 \pm 10\%$) on 12/12/ light/dark period. The animals were provided ad libitum access to food and water.

Treatment; the subjects were allocated into two groups and sixteen subjects were included in each group. Subjects of Group 1 were injected with 120 mg/kg propofol (Fresenius, France) and subjects of Group 2 were injected with same dose of intralipid (Fresenius, France).

Procedure; all the injections were given at Zeitgeber Time 10 (ZT10), ten hours after light on and the subjects were sacrificed after euthanasia under CO₂ at day 1 and day 3 at ZT5 (five hours after light on). Blood was collected, serum samples were prepared and stored at -20 °C. A morphine specific enzyme-linked immunosorbent assay kit (Immunalysis Corporation, USA) was used to quantify the amount of endogenous morphine. For all the samples, endogenous morphine standards were used and coefficient of variance (CV) were less than 8%. Any sample with a CV more than 8% was retested to obtain appropriate CV.

RESULTS

One way ANOVA showed a significant effect between treatments: propofol and intralipid control when

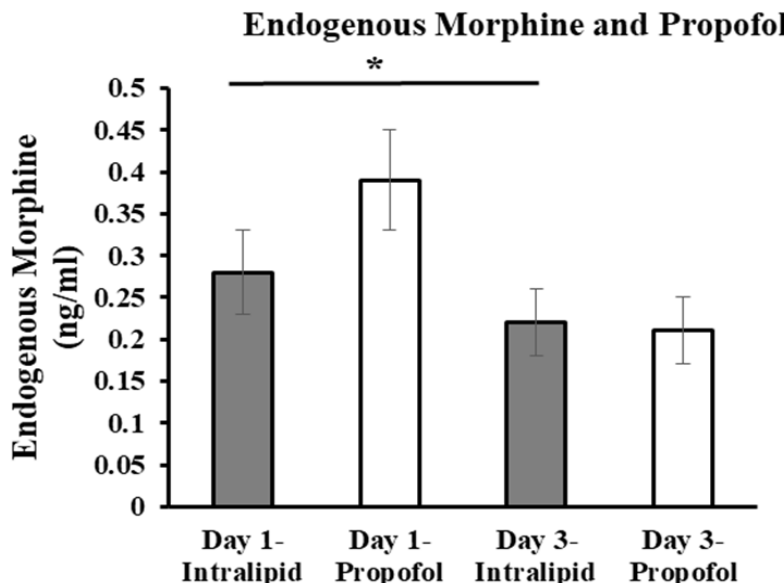


Figure 1: Endogenous morphine (ng/ml) in rats' serum after propofol anesthesia (* $p < 0.05$).

subjects were sacrificed at day 1 ($F(1, 11) = 85.66$, $p = 0.001$). There was also a significant effect of the day (between day 1 and day 3) of treatment with propofol determined by one way ANOVA ($F(1, 10) = 7.034$, $p = 0.02$). However, there was no significant effect of the day of treatment with intralipid determined by one way ANOVA ($F(1, 10) = 0.739$, $p = 0.41$). Results are shown in Figure 1.

DISCUSSION

The current study investigated that there is significant effect of short duration anesthesia propofol on endogenous morphine when subjects were sacrificed a day after the treatment. There was also significant effect of the day of treatment with propofol. However, there was no effect of intralipid either given at day 1 or day 3. For the quantification of endogenous morphine, Sprague dawley rats were used, as these rodents have been shown to have endogenous morphine in the blood samples.⁸ The levels of endogenous morphine were quantified by using enzyme-linked immunosorbent assay, as this technique is useful for the quantification of secondary metabolites like morphine.⁹

The subjects were sacrificed at ZT5 to synchronize the experiments, as different proteins show circadian variation during 24 hours of the day. However, no circadian variation of endogenous morphine is studied yet, although endogenous morphine down regulates different proteins and such proteins show circadian variations.¹⁰ Subjects received intralipid as controls to avoid any bias of lipid effect¹¹, as propofol anesthesia is lipophilic and is in lipidic solution.

effect of propofol on endogenous morphine

The current study highlights for the first time the effect of any anesthetic on endogenous morphine which was also being used as anesthetic and analgesic. Propofol showed an effect on endogenous morphine that is increased at day 1. This might be due to the fact that as propofol injections are locally painful,¹² the pain may trigger the endogenous morphine release as a reaction to provide analgesic effect.

This effect might continue for around 48 hours, as on the day 3, endogenous morphine was observed to be equal to that in intralipid controls. This may be due to any circadian effect, as different anesthetic effects have been observed at different times of the day¹³ and it needs to be investigated further. Whatever the reason of the increase of the endogenous morphine, it may have protective effects, as opioid induced cardioprotection has been observed against myocardial infarction.¹⁴

A recent study showed cardiac protection by suppressing proteasome degradation of caveolin-3 in ischemic rodents.¹⁵ For anesthesiologists, the use of propofol may be more protective as it gives synergistic effect by increasing endogenous morphine. Especially, this may enhance the use of propofol anesthesia with cardiac procedures and off course, further studies are needed to be conducted.

LIMITATIONS

Few limitations of the study: this increase of the endogenous morphine is limited to only short term

propofol anesthesia and in young rats at zeitgeber time 5. Two questions remain to be answered; whether the results of this and similar studies can be applied to human beings and whether control of injection pain with propofol will have a dampening effect on the level of released endogenous morphine.

CONCLUSION

We conclude that there is a positive effect of propofol anesthesia in increasing endogenous morphine levels, and it might be implicated in cardioprotection and a possible chrono-therapeutic effect which should be considered for further studies. However, this may be limited to short duration anesthesia and or only propofol anesthesia at ZT5. So, this may not predict any effect of long duration anesthetics and/ or other zeitgeber times.

Compliance with ethical standards

All the procedures were performed at Institute of Cellular and Integrative Neurosciences Strasbourg, France. Ethics on animal care used were followed in compliance with Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, France.

Conflict of interest: Author declare that there is no conflict of interest for the current study.

Authors' contribution:

MR: Concept, experiments, writing

LP: Concept, manuscript editing

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