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Effects of Medetomidine on Analgesia and Sedation in Rats

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Abstract : The effects of medetomidine on the degree of analgesia and sedation in rats were evaluated. The rats were randomly divided into six groups: saline, 1 mL/kg (group 'Saline'); butorphanol, 2.0 mg/kg; medetomidine, 0.2, 0.4, 0.8 or 1.6 mg/kg (group 'MED0.2', 'MED0.4', 'MED0.8' and 'MED1.6', respectively). The degree of analgesia was measured in the 50°C hot-water tail-flick latency test, and the degree of sedation was evaluated using the numerical sedation score (NSS) and the righting reflex. All doses of medetomidine, except MED0.2, significantly increased the analgesic effect compared to the Saline group. Variables in the MED0.4 and MED0.8 groups, but not in the MED1.6 group, were significantly increased compared to those in the MED0.2 group. However, analgesia with all doses of medetomidine was not significantly different compared to that with butorphanol. Saline and butorphanol treatments did not induce sedation and loss of righting reflex during the recording period. NSS in the MED0.4, MED0.8 and MED1.6 groups were significantly higher than that in the MED0.2 group. NSS in the MED0.8 and MED1.6 groups were not significantly different from that in the MED0.4 group. The latency to loss of righting reflex in the MED0.8 and MED1.6 groups decreased significantly compared to that in the MED0.2 group. Thus, 0.4 and 0.8 mg/kg of medetomidine provided not only reliable analgesia but also sedation to rats. In conclusion, 0.4 to 0.8 mg/kg medetomidine could be a useful chemical restraint method in rats.

Key words **:** medetomidine, tail-flick test, analgesia, sedation, rat.

Introduction

Alpha$_2$-adrenoceptor agonists produce a firm sedative effect and dose-dependent analgesia by stimulating presynaptic α_2-adrenoceptors (1,5,7,10,17,24). The sedative and analgesic effects are dependent on which supraspinal and spinal sites of α_2-adrenoceptors are activated (4,8). Medetomidine is a highly selective α_2-adrenoceptor agonist, and is used as a sedative and pre-anaesthetic in various species including rats (11,12,14,15, 20,25). Although rare reports have introduced medetomidine for chemical restraints in rats, medetomidine has some advantages; its sedation and analgesic effects are introduced simultaneously, and medetomidine has a selective antagonist, atipamezole, which can be used to control medetomidine efficacy.

In most rat experiments, general anesthesia is applied minor pain can be processed and conducted in a relatively short period, but sedation is needed to restrain the rats.

Previous results demonstrated that dexmedetomidine, an α_2-adrenoceptor agonist, produced a dose-dependent increase of sedation in cats, but interestingly only the highest doses resulted in analgesia (23). Similarly, dexmedetomidine exerts an analgesic effect at high plasma concentration in rats during target-controlled infusion (3). However, marked variation in sensitivity to each α_2-adrenoceptor agonist is seen between species, and the duration of action, sedative and analgesic effects of each individual drug also vary (17).

In the present study, we evaluated the analgesic and sedative effects of different doses of medetomidine in rats.

Materials and Methods

Experimental animals

Thirty-two adult male Sprague-Dawley rats (Samtaco, Osan, Korea) weighing 260-400 g were used. Each two animals were housed in a Plexiglas cages (28 cmW × 42 cmD × 18 cmH) with standard bedding. They were maintained under controlled environments throughout the study: 22-24°C ambient temperature, relative humidity 40-45%, 12:12-hr light-dark cycles (lights on from 7:00 to 19:00), and a commercial pelleted chow (Hyochang Science, Daegu, Korea) and tap water available *ad libitum* except on the day of the experiment. The health of the animals was clinically checked by the veterinary staff and body weight was measured every day including the day of the experiment. If loss of body weight or symptom of disease were observed, rats were excluded from study. The rats were euthanized by the overdose of CO_2 inhalation after the completion of experiment. The experiments were approved by the Kyungpook National University Institutional Animal Care and Use Committee, and carried out in accordance with the National Institutes of Health Guidelines for the Care and

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Use of Laboratory Animals (1996).

Drugs

Medetomidine (Domitor®, Orion Corporation Animal Health, Turku, Finland), normal saline and butorphanol (Butophan®, Myungmoon Pharm. Co. Ltd., Seoul, Korea) were purchased a commercial form of product. Drugs were administered intraperitoneally in the present study, unless other methods were stated. Volume for each drug is different; 1.0 ml/kg for normal saline and butorphanol, and 0.2, 0.4, 0.8 and 1.6 ml/kg for 0.2, 0.4, 0.8 or 1.6 mg/kg of medetomidine.

The degree of analgesia

Thermal anti-nociception was assessed using hot-water tail-flick latency (TFL) (13,19). The thermal stimulus comprised immersion of one- to two-thirds of the tail in a 50°C water bath. The immersed tail length was alternated to avoid sensitization or adaptation. During testing, the rats were loosely wrapped in a towel and gently restrained by the experimenter. Only tail flicking was admitted as a response, and the latency to tail-flick response was measured with a stopwatch. The cut-off time was set at 15 sec to minimize tissue damage. After each measurement, the tail was dried with a paper towel. TFLs are expressed as a percentage of the calculated maximal possible effect (%MPE).

%MPE = (post-drug latency (s) – pre-drug latency (s) / cut-off value (15 s) – baseline latency (s)) × 100

The degree of sedation

Numerical sedation score (NSS) and the latency to loss of righting reflex were measured. NSS was measured to assess the degree of sedation and immediately before the TFL test. The NSS was on a scale from 0 to 5: 0, no sedation; 1, less alert but still active; 2, drowsy, but still show righting reflex and walking spontaneously or upon gentle knocking at the cage; 3, very drowsy, dorsal or lateral recumbent but walking or moving upon taking the lid off the cage or during the towel wrapping procedure; 4, lateral recumbent, but little struggle after towel wrapping; 5, lateral recumbent and no response to the towel wrapping procedure. To assess NSS, the rats were initially monitored undisturbed and unrestrained in the cage. When the rats showed no response, the experimenter gently knocked two or three times on the outside of the Plexiglas cage. The lid of cage was gently removed, the righting reflex was briefly examined, and the rats were then loosely wrapped in a towel for the TFL test.

The latency to loss of righting reflex was calculated as the time to first onset of loss of righting reflex after medetomidine injection. The experimenter gently grasped and turned the rat to ventral recumbency every 1-min after medetomidine injection whether the rat showed a righting reflex or not. If a rat was still active or intensely struggled to escape, the test was passed to the next test time. After the rat showed loss of righting reflex, it was positioned at lateral recumbency.

Experimental procedures

The rats were randomly divided into six groups ($n = 9$ for each group): saline, 1 ml/kg (group 'Saline'); butorphanol, 2.0 mg/kg (group 'BUT'); medetomidine, 0.2, 0.4, 0.8 or 1.6 mg/kg (group 'MED0.2', 'MED0.4', 'MED0.8' and 'MED1.6', respectively). The rats participated in two trials with a > 7-days washout interval, and we made every effort to include the rats in different trials.

On the day of the experiment, the rats were allowed to acclimate for > 1 hr in a Plexiglas cage in the recording room. Thirty min before the baseline measurements, for the evaluation of the warm allodynia, the TFL test was performed in warm water (40°C), (2,21) and only the rats that showed 30-s cut-off of TFL in this test were included in further studies. Baseline TFL was measured three times at 30-min intervals. The third baseline value measurement was finished by twelve o'clock. Drug treatment was always begun between 14:00 and 14:15 to minimize any errors introduced by circadian rhythm. Post-drug TFL and NSS were measured at 30-min intervals for 2 hr from completion of drug treatment.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Variables of tail-flick test and NSS were analyzed by two-factor repeated measures ANOVA with time and treatment, and a Bonferroni test was used to analyze variables for time between groups (SPSS 14.0K; Datasolution, Seoul, Korea). The latency to loss of righting reflex between groups was compared using a one-way ANOVA and Bonferroni test. A value of $p < 0.05$ was considered statistically significant.

Results

The degree of analgesia (Fig 1.)

Repeated measures ANOVA revealed that drug administration ($F = 12.45$, $p < 0.001$) and time course ($F = 7.73$, $p < 0.001$) significant affected %MPE. Bonferroni test indicated that all doses of medetomidine and butorphanol treatments, except of MED 0.2 mg/kg treatment group ($p = 0.059$), significantly increased %MPE ($p < 0.001$ in all groups) when compared to Saline group. %MPE of MED0.4 and MED0.8 groups significantly increased ($p = 0.047$ and $p = 0.009$, respectively) when compared to MED0.2 group, but not in the MED1.6 group ($p = 1.000$). However, all doses of medetomidine treatments did not significantly increased %MPE when compared to butorphanol treatments.

The degree of sedation (Fig 2.)

Saline and butorphanol treatments did not induce sedation during the recording period. Repeated measures ANOVA revealed that drug administration ($F = 123.11$, $p < 0.001$) and time course ($F = 26.40$, $p < 0.001$) significantly increased NSS. Bonferroni test showed that butorphanol treatment did not significantly changed NSS when compared to saline treatment. All dose of medetomidine significantly increased NSS when compared to

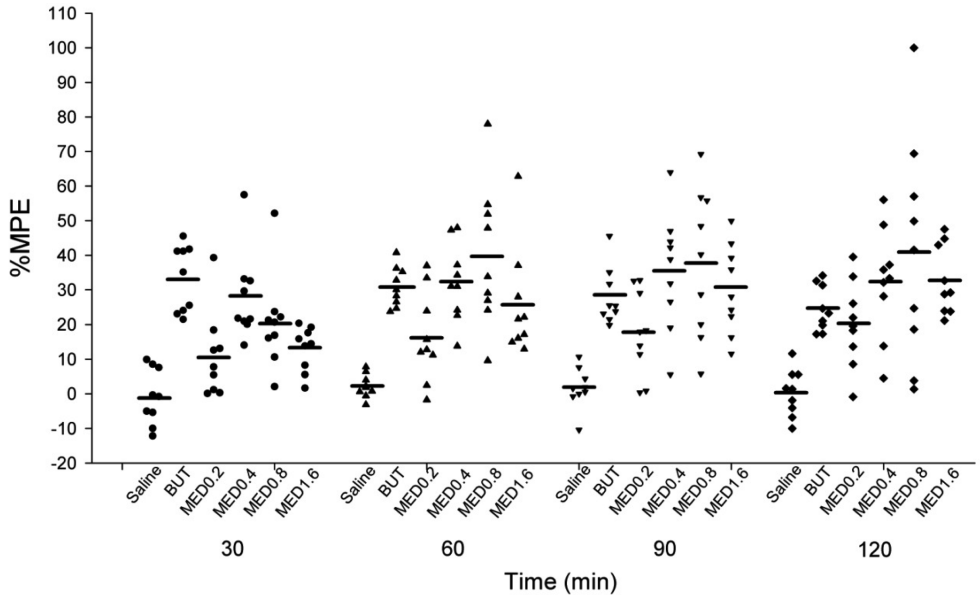

Fig 1. Time course of %mean possible effect (%MPE) of tail-flick latency using 50°C water before and 30, 60, 90 and 120 min after intraperitoneal saline (Saline group), butorphanol (BUT group), or 0.2, 0.4, 0.8 and 1.6 mg/kg of medetomidine injection (MED0.2, MED0.4, MED0.8 and MED1.6 group, respectively) in rats. Although data were analyzed by two-factor repeated measures ANOVA followed by a Bonferroni test, statistical results were not marked in figure but were described in text. Each dot indicates the data at each recording time in each rat, and horizontal line indicates the mean value, and $n = 9$/group.

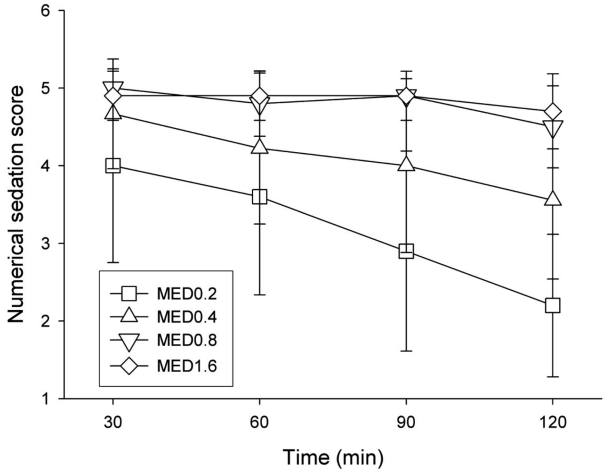

Fig 2. Time course of numerical sedation score (NSS) in rats treated with intraperitoneal saline (Saline group), butorphanol (BUT group) or 0.2, 0.4, 0.8 or 1.6 mg/kg of medetomidine (MED0.2, MED0.4, MED0.8 and MED1.6 group, respectively). NSS was ranged from 0 (no sedation) to 5 (the deepest sedation). Although data were analyzed by two-factor repeated measures ANOVA followed by a Bonferroni test, statistical results were not marked in figure but were described in text. Data were expressed as mean ± standard deviation, and n = 9/group.

Saline and BUT groups ($p < 0.001$). NSS in MED0.4, MED0.8 and MED1.6 groups significantly increased NSS ($p = 0.025$ in MED0.4 group, $p < 0.001$ in MED0.8 and MED1.6 groups) when compared to MED0.2 group. MED0.8 and MED1.6 group did not significantly increase NSS when compared to MED0.4 group ($p = 0.653$ in MED0.8 group and $p = 0.529$ in MED1.6 group).

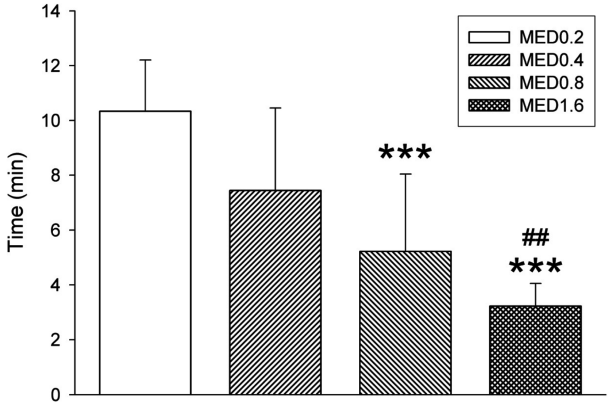

Fig 3. Latency to loss of righting reflex in rats after treatment with intraperitoneal 0.2, 0.4, 0.8 or 1.6 mg/kg of medetomidine injection (MED0.2, MED0.4, MED0.8 and MED1.6 group, respectively). Data were expressed as mean ± standard deviation, and $n = 9$/group. ***indicates a significant difference from MED0.2 group ($p < 0.001$), and ##indicates a significant difference from MED0.4 group ($p < 0.01$).

The latency to loss of righting reflex (Fig 3.)

The rats in the Saline and BUT groups and one rat in the MED0.2 group did not show loss of righting reflex; therefore, their data were not included in the statistical analysis. One-way ANOVA revealed that medetomidine treatments significantly affected the latency to loss of right reflex ($F = 15.85$,

$p < 0.001$). Bonferroni test demonstrated that latency in the MED0.8 and MED1.6 groups, but not in the MED0.4 group ($p = 0.072$), significantly decreased ($p < 0.001$ in both) when compared to MED0.2 group. The onset of the loss of righting reflex in MED1.6 group was significantly reduced ($p = 0.003$) when compared to MED0.4 group.

Discussion

The present results show that all doses of medetomidine produce sedative effects, and can also induce loss of righting reflex in rats. In contrast, the analgesic effect is reliable when a dose of at least 0.4 mg/kg of medetomidine is administered to rats.

The present results are similar with that an α_2-adrenoceptor agonist dose-dependently prolongs sedation without increasing analgesia (17). Although the duration of sedation was not measured, the present results show that the sedative effects are dose-dependent.

The present results are somewhat different from previous results in which only high-dose dexmedetomidine provided analgesia in cats (23), and analgesic effects were exerted at high plasma concentration (3). Zhang and co-workers demonstrated that intraperitoneal dexmedetomidine significantly and dose-dependently increased the paw withdrawal latency (PWL) at the affected limb in a rat model of monoarthritis (26). However, this result was somewhat different at the contralateral intact paw; only the highest dose transiently increased PWL. That is, dexmedetomdine also has a similar analgesic effect in the rats. Although difficult to conclude, the differences in the metabolic mechanism between species, the drugs and the degree of noxious stimuli could be reasons for the apparent differences in the results. First, one study was performed in cats while we used rats. In case of the drugs, to our knowledge, there is no report comparing the effect of medetomidine and dexmedetomidine in the same species, and further study is needed. Second, thermal antinociception testing method was different, and each could involve different analgesic effects; 50°C hot-water tail immersing test and tail-flick latency time was measured in the present study. Heat source by heat device on the thorax and heat increased to cut-off of 55°C, and response at threshold temperature was recorded in the previous study.

The rats in the MED1.6 group showed no significant difference in %MPE compared to the MED0.2 group, and variables in MED0.8 were highly deviated. That is, the present result clearly showed that the analgesic effect of medetomidine is not increasing in a dose-dependent manner, but rather becomes more variable and unreliable. Although the explanation is hard to find, it is possible that surplus efficacy of the high dose of the α_2-adrenoceptor agonist, medetomidine, includes the activation of α_1-adrenoceptors. Actually, Millan (18) stated in his review that the degree of residual activity of centrally-active α_2-adrenoceptor agonist analgesics at α_1-adrenoceptor is a crucial issue. That is, 0.8 and 1.6 mg/kg of medetomidine could have residual efficacy on α_1-adrenoceptors, such that activation of α_1-adrenoceptors will counter the sedative effects of the α_2-adrenoceptor agonist and can raise the descending facilitation and behavioral hyperalgesia (6,9,16).

Slingsby and co-workers indicated that sedation might affect analgesia related value, because of confounded signs of analgesia with sedation, but they did not observe the that phenomenon in their study (22). In the present study, sedation by medetomidine also does not appear to affect an evaluation of analgesia, because the mean values of %MPE were not in direct proportion to NSS, and %MPE in MED1.6 group is rather less than MED0.8 group.

Thus, 0.2 mg/kg of medetomidine could not provide reliable analgesic effects, but 0.4 and 0.8 mg/kg of medetomidine presented significantly higher analgesic effects than saline and 0.2 mg/kg of medetomidine. All medetomidine groups produced significantly enhanced analgesic and sedation potency compared to the MED0.2 group, but not compared to the MED0.4 group. That is, 0.4 and 0.8 mg/kg of medetomidine simultaneously provide reliable sedation and analgesia to rats. In conclusion, 0.4 to 0.8 mg/kg of medetomidine could be a useful chemical restraint method in rats.

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Medetomidine의 투여가 흰쥐의 진통과 진정효과에 미치는 영향

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요 약 : Medetomdine의 투여가 흰쥐의 진통과 진정효과에 미치는 영향에 대하여 평가하였다. 실험동물은 생리식염수 1 ml/kg 투여군 (group 'Saline'), butorphanol, 2.0 mg/kg 투여군 (group 'BUT'), medetomidine, 0.2, 0.4, 0.8 or 1.6 mg/kg 투여군 (각각 group 'MED0.2', 'MED0.4', 'MED0.8' and 'MED1.6') 등 6개의 실험군으로 나누어졌다. 진통효과는 50°C hot-water tail-flick latency test로 측정하였고, 진정효과는 numerical sedation score (NSS)와 정위반사 (righting reflex)로 평가하였다. MED0.2군을 제외한 모든 medetomidine 투여군들이 Saline군에 비해 유의적인 진통효과의 증가를 보였다. MED0.2군과 비교할 때 MED0.4와 MED0.8군은 유의적인 진통효과의 상승을 보였으나, MED1.6군은 유의적인 변화를 보이지 않았다. BUT군과 모든 medetomidine 투여군은 진통효과에서 유의적인 차이를 보이지 않았다. Saline과 butorphanol의 투여는 진정효과와 정위반사의 소실을 유발하지 않았다. Medetomidine의 투여용량의 증가는 진정효과를 증가시켰다. MED0.2군과 비교할 때 MED0.4, MED0.8과 MED1.6군의 NSS가 유의적으로 증가하였으나, MED0.4군에 비해서는 MED0.8과 MED1.6군이 유의적인 차이를 나타내지 않았다. 정위반사의 소실 시간은 MED0.2군에 비해 MED0.8과 MED1.6군이 유의적으로 감소하였다. 본 실험의 결과로 볼 때, 흰쥐에서 medetomidine 0.4에서 0.8 mg/kg 이내의 용량을 복강내 투여할 때 신뢰할 수 있는 진통효과와 진정효과를 동시에 얻을 수 있으며, 따라서 흰쥐의 진정, 진통 및 화학적 보정 목적에 적절한 용량임을 알 수 있었다.

주요어 : 메데토미딘, tail-flick test, 진통효과, 진정효과, 흰쥐