ORIGINAL ARTICLE

Ephedrine enhances the antinociceptive effect of dexmedetomidine in mice

Zafer Sezer, Gülay Sezer, Yalçın Tekol

Erciyes University, Faculty of Medicine, Department of Pharmacology, Kayseri, Turkey

Correspondence to: Dr. Zafer Sezer
Erciyes University, Faculty of Medicine, Department of Pharmacology
38039 Kayseri, Turkey.
TEL: +90 352 4374937 (24417); FAX: +90 352 4374067;
E-MAIL: sezerza@yahoo.com, zsezer@erciyes.edu.tr

Submitted: 2010-08-26 Accepted: 2011-07-10 Published online: 2011-08-29

Key words: **antinociception; dexmedetomidine; ephedrine; hot plate; mice**

Neuroendocrinol Lett 2011; **32**(4):552–556 **PMID:** 21876510 NEL320411A19

Abstract

OBJECTIVES: Dexmedetomidine, a highly selective alpha-2-adrenoceptor agonist, was recently introduced into clinical practice for its sedative and analgesic properties. The purpose of this study was to evaluate whether the psychostimulant drug ephedrine has any effect on dexmedetomidine-induced antinociception and locomotor inhibitor activity in mice in acute application.

METHODS: In both sexes of swiss albino mice; antinociception was assessed with hot-plate test and the locomotor, exploratory activities were assessed with holed open field test. The animals were received; saline + saline, ephedrine (10 mg/kg) + saline, saline + dexmedetomidine (15 μg/kg) and ephedrine (10 mg/kg) + dexmedetomidine (15 μg/kg), intraperitoneally, 30 min before hot plate or holed open field tests.

RESULTS: In the hot plate test in mice, co-administration of 15 μg/kg dexmedetomidine with 10 mg/kg ephedrine intraperitoneally not only enhanced, but also prolonged the duration of antinociception induced by dexmedetomidine. At the same time, the locomotor inhibitory effect of dexmedetomidine was counteracted by ephedrine.

CONCLUSION: We concluded that the combined administration of dexmedetomidine with ephedrine may have beneficial effects in the treatment of pain without causing sedation, which limits the use of dexmedetomidine as an analgesic in humans.

INTRODUCTION

It is well established that alpha-2 (α_2-) adrenergic receptor agonists produce analgesia and sedation, and they are used in the postoperative phase or in intensive care as sedative, hypnotic and analgesic agents (Kamibayashi & Maze 2000; Sandler 1996).

α_2-Adrenoceptors are located in the brain, spinal cord and periphery and three subtypes have been identified in humans and mice, i.e. α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors (Bylund *et al.* 1994; Philipp *et al.* 2002). α_2-Agonists mediate sedation in the locus ceruleus (Nelson *et al.* 2003) and produce analgesia by action at several sites including supraspinal, spinal and peripheral sites. The spinal cord is reported to be the principle site of the analgesic or analgesic enhancement effects of α_2-adrenergic receptor agonists; however, analgesic action has also been reported in supraspinal and peripheral sites (Pertovaara *et al.* 1991; Poree *et al.* 1998).

Dexmedetomidine has a relatively high ratio of α_2/α_1–activity (1620:1 as compared with 220:1 for

clonidine) (Virtanen *et al.* 1988) and it has negligible effects on imidazoline receptors. This may result in stronger sedative effects without any of the unwanted cardiovascular effects which arise from α_1-receptor activation (Hall *et al.* 2000). Dexmedetomidine, like clonidine, has been found to reduce the need for general anesthetics and analgesics in patients (Aantaa *et al.* 1990). In a study with transgenic mice have shown that both dexmedetomidine-induced analgesia and sedation are mainly mediated by the α_{2A}-adrenoceptor subtype (Hunter *et al.* 1997).

Ephedrine is a central nervous system stimulant which has a pharmacological profile similar to amphetamines (Jacobs & Hirsch 2000). It has been shown that, like amphetamines, ephedrine is also able to potentiate morphine-induced analgesia in mice (Dambisya *et al.* 1990, 1991). In one study the analgesic effect of morphine-ephedrine combination was compatible with the effect of morphine given alone in a double dose (Tekol *et al.* 1994).

There has been increasing research in recent years on drug combinations that produce analgesia in experimental animals and these may be useful in clinical populations. The general purpose of drug combinations is to enhance analgesia by synergism and/or to reduce drug side effects by either reducing the dose of each drug or allowing drugs to interact. As the clinical use of α_2-adrenoceptor agonists for pain relief is restricted by side effects such as sedation and hypotension (Sandler 1996), we conducted this study in order to see whether the psychostimulant drug ephedrine has any effect on dexmedetomidine-induced antinociception and locomotor inhibitory activity in mice in acute application.

METHODS

Experiments were performed in accordance with the protocol approved by Erciyes University's Medical Faculty's Ethical Committee for Animal Experimentation. All testing was performed in accordance with the policies and recommendations of the International Association for the Study of Pain (Zimmermann 1983).

Experiments were conducted on adult Swiss albino (weighing 25–35 g) mice from our own breeding facilities and each group consisted of an equal number of male and female animals (n=8). Except during experiments, the animals were allowed free access to food (Aytekinler standard pellets, Turkey) and tap water and were kept under artificial light for 12 h/day (lights on at 7.00 a.m.) in a room with controlled temperature (22±2 °C) and humidity (50±10%).

The mice were tested with a hot plate analgesia meter (MAY 9601, Turkey) which was set at 52.5± 0.1 °C. Each animal was tested on the hot plate before treatments (baseline latency) and at 30, 60, 90 minutes (min) after injections (postdrug latencies). Baseline latencies were between 10–20 seconds (s). The end point was determined by paw withdrawal, hind paw licking or jumping, and to prevent tissue damage, the cut-off time was set at 45 s.

Hot plate latencies were converted to percentage maximal possible effect (% MPE) by the following equation (Wesolowska 2004); % MPE= (Postdrug Latency-Baseline Latency) / (Cut off Latency-Baseline Latency) × 100

Locomotor and exploratory activities were assessed in another group of mice by the holed open field test (Weischer 1976). The apparatus consisted of an octagonal, grey painted, open-topped wooden box measuring 14 × 20 × 1 cm (width × depth × thickness) with 16 holes (2 cm diameter and with a 3 cm distance between the holes) on the walls. The floor of the arena was divided into eight equally spaced rectangles. The mice were placed in the centre of the board one by one and left to move freely. The number of parts crossed with all paws (locomotor activity) and the number of head dips into the holes (exploratory) were counted in 5 min sessions 30 min after treatments.

Mice were (n=8) injected with saline + saline, ephedrine (10 mg/k g) + saline, saline + dexmedetomidine (15 μg/kg) and ephedrine (10 mg/kg) + dexmedetomidine (15 μg/kg) 30 min before hot plate or holed open field tests. All drugs were freshly prepared by dilution of the injectable forms of drugs with saline and injected intraperitoneally (i.p) in a total volume of 10 ml/kg. In experiments, ephedrine HCl (Biosel Pharmaceuticals, Turkey) or saline was injected 5 minutes before the dexmedetomidine HCl (Precedex®, Abbott Labs, USA) and doses refer to the salt weight of the drugs.

Data were tested for normality and homogeneity of variance prior to parametric analysis. For the hot plate test, differences between groups and also according to times were assessed by a repeated measure two-way analysis of variance (ANOVA) and followed by a post hoc Tukey test for multiple comparisons. Locomotor and exploratory activites were evaluated by using one-way ANOVA and followed by Tukey test for post hoc comparisons. Results were expressed as means ± standard error of the mean (S.E.M.) and the level of significance was set at a p-value <0.05 for all data.

RESULTS

Analysis of variance indicated that significant differences between groups (F =32.915, p<0.0001) and also within groups (F =26.082, p<0.0001) according to time (F =6.407, p<0.0001) on % MPE in mice. Dexmedetomidine showed significant antinociceptive effect at 15 μg/kg at 30 min (p<0.05) and the effects at 60 and 90 min were not different from those of the saline treated group (p>0.05). On the other hand ephedrine induced antinociceptive effect at 10 mg/kg i.p. only at 60 min in mice (p<0.05). In the ephedrine+dexmedetomidine combined group antinociception was statistically significant at 30, 60 and 90 min compared to the saline,

dexmedetomidine or ephedrine administered groups ($p<0.05$). Results are presented in the Figure 1.

There were singnificant differences on the locomotor (F=36.138, $p<0.0001$) and exploratory (F=16.125, $p<0.0001$) activities between the groups. Dexmedetomidine inhibited locomotor and exploratory activities significantly compared to the saline, ephedrine and ephedrine+dexmedetomidine treated groups ($p<0.05$). On the contrary, ephedrine stimulated just locomotor activity significantly ($p<0.05$). The exploratory activity of the ephedrine injected animals was similar with saline injected group. In the ephedrine+dexmedetomidine combined group locomotor activity and exploration were not significantly different from the saline treated group ($p>0.05$). Results are given in the Figure 2.

DISCUSSION

The findings of the present study show that co-administration of ephedrine with dexmedetomidine a) enhanced the antinociceptive effect of dexmedetomidine b) prolonged the duration of antinociception induced by dexmedetomidine and c) reduced the locomotor inhibitory effect of dexmedetomidine.

In acute (Asano *et al.* 2001; Buerkle & Yaksh 1998) and chronic pain models (Malmberg *et al.* 2001; Poree *et al.* 1998), clonidine and dexmedetomidine have been seen to produce profound antinociception in rodents. In addition, dexmedetomidine and clonidine were found to reduce the need for general anaesthetics and analgesics in patients undergoing surgeries (Aantaa *et al.* 1990; Quintin *et al.* 1996). The most common use of α_2-adrenoceptor agonists, such as analgesics and/or anaesthetic sparing agents, has been restricted to either epidural or spinal administration. However, while effective analgesia with less pronounced side effects is obtained by these restricted routes of administration, widespread use of α_2-adrenoceptor agonists for the treatment of pain following systemic administration is precluded by a series of adverse side effects including sedation, profound systemic hypotension and bradycardia (Chrysostomou & Schmitt 2008; Kamibayashi & Maze 2000). In our study we combined ephedrine with dexmedetomidine in order to investigate whether it affects the antinociceptive effect and locomotor inhibitor activity of dexmedetomidine after systemic administration. We used relatively small doses of dexmedetomidine (15 µg/kg) to induce antinociception and very little sedation so as not to significantly affect the behavior of the animals during hot-plate test. Dexmedetomidine induced antinociception was only significant at 30 min in our study. Dawson *et al.* (2006) showed that the antinociceptive effect of 12.5 µg/kg i.p. dexmedetomidine peaked between 20 and 30 min after administration and was sustained for up to 90 min in tail-flick test in rats. Although some previous studies have shown that ephedrine had no demonstrable antinociceptive effects in the tail flick test and tail immersion assays at 5, 10 and 20 mg/kg doses (Dambisya *et al.* 1990; 1991; Tekol *et al.* 1994), in our study it produced antinociception at 10 mg/kg, 60 min after administration. The differences between the results of studies may depend on kind of tests, animals and doses of drugs. In the present study, ephedrine administration not only enhanced, but also prolonged the duration of antinociception induced by dexmedetomidine for 90 min in the hot-plate test. In addition, the inhibition produced by dexmedetomidine on locomotor and exploratory activities were counteracted by ephedrine. Similar to our results, combination of clonidine and ephedrine effec-

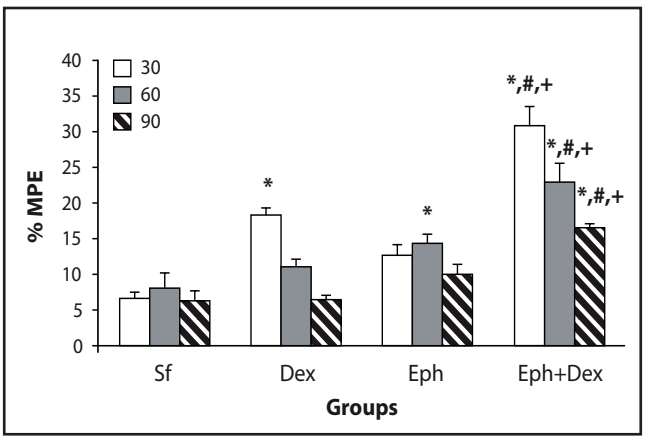

Fig. 1. The effects of saline (Sf), dexmedetomidine (Dex, 15 µg/kg), ephedrine (Eph, 10 mg/kg) and ephedrine + dexmedetomidine (Eph+Dex, 10 mg/kg+15 µg/kg) administration (i.p.) in hot plate test at 30, 60 and 90 minutes in mice. Latencies were converted to percentage of maximal possible effect (% MPE). Each column represents the mean values of 8 mice.Vertical bars indicate S.E. of mean. *p<0.05* as compared to saline (*), dex (#) and eph (+) treated groups.

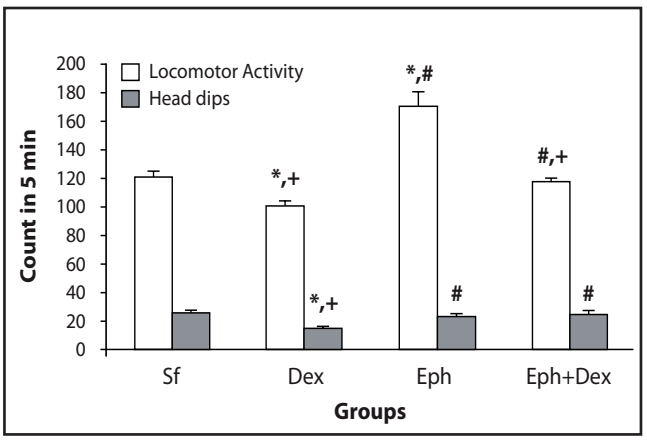

Fig. 2. The effects of saline (Sf), dexmedetomidine (Dex, 15 µg/kg), ephedrine (Eph, 10 mg/kg) and ephedrine + dexmedetomidine (Eph+Dex, 10 mg/kg+15 µg/kg) administration (i.p.) on the locomotor activitiy and exploratory behavior in mice tested in the holed open field test. Each column represents the mean of 8 mice and vertical bars indicate S.E. of mean. *p<0.05* as compared to saline (*), dex (#) and eph (+) treated groups

tively reduced propofol-induced pain in humans (Ishiyama *et al.* 2006). Although the cardiovascular effects of this combination were not assessed in the present study, based on the pharmacological properties of the drugs, combining dexmedetomidine with ephedrine may inhibit the hypotensive effect of dexmedetomidine. It was shown that ephedrine potentiated the antinociceptive effects of opioids in mice and humans (Dambisya *et al.* 1990; 1991; Tekol *et al.* 1994) and also combination with morphine diminished some of the well-known side effects of morphine such as hypotension, respiratory depression, nausea, vomiting, dizziness and sedation in postoperative patients (Tekol & Koramaz 1996).

Dexmedetomidine has been shown to produce antinociception by an action at several sites; including spinal (Kalso *et al.* 1991) and supraspinal level by α_2-adrenoceptors. It has been shown that the antinociceptive effect of intraperitoneal dexmedetomidine was blocked by pretreatment with intraperitoneal atipamezole, a selective α_2-adrenoceptor antagonist, in rats (Guo *et al.* 1996). It is well known that ephedrine has direct agonistic activity at α_2-adrenergic receptors, it also enhances the release of endogenous norepinephrine from axon terminals (Jacobs & Hirsch 2000), which has been shown to act on postsynaptic sites to reduce noxious stimuli through α_2-adrenoceptors (Pertovaara 2006). Similarly, Dambisya *et al.* (1991) reported that ephedrine potentiated the antinociceptive effects of morphine through its effect on α_2-adrenoceptors as the potentiation was effectively antagonised by yohimbine. Although we did not attempt to clarify the action mechanism of the dexmedetomidine+ephedrine combination on antinociception and locomotor activity, there may be a synergistic and antagonistic interaction between dexmedetomidine and ephedrine on pain relief and locomotor activity, respectively.

As the analgesic and locomotor inhibiting/sedative responses to α_2-adrenoceptor agonists seem to be mainly mediated through the activation of α_{2A}-adrenoceptors (Hunter *et al.* 1997), it is difficult to find an α_2-adrenoceptor agonist with an acceptable analgesic activity which does not cause sedation following systemic administration. For this reason, combined treatment is a more conceivable route in the search for enhanced pain relief and reducing the side effects of α_2-adrenoceptor agonists. The present study shows that ephedrine pretreatment may have beneficial effects in the treatment of pain with dexmedetomidine without causing sedation which limits the use of the drug as an analgesic in humans. However, further clinical studies are needed in order to confirm this interaction.

REFERENCES

1 Aantaa R, Kanto J, Scheinin M, Kallio A, Scheinin H (1990) Dexmedetomidine, an alpha-2-adrenoceptor agonist, reduces anesthetic requirements for patients undergoing gynecologic surgery. Anesthesiology. **73**: 230–235.

2 Asano T, Dohi S, Ohta S, Shimonaka H, Iida H (2000) Antinociception by epidural and systemic α_2-adrenoceptor agonists and their binding affinity in rat spinal cord and brain. Anesth Analg. **90**: 400–407.

3 Buerkle H, Yaksh TL (1998) Pharmacological evidence for different alpha$_2$ adrenergic receptor sites mediating analgesia and sedation in the rat. Brit J Anaesth. **81**(2): 208–215.

4 Bylund DB, Eikenberg DC, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP *et al.* (1994) IV. International union of pharmacology nomenclature of adrenoceptors. Pharmacol Rev. **46**(2): 121–136.

5 Chrysostomou C, Schmitt CG (2008) Dexmedetomidine: sedation, analgesia and beyond. Expert Opin. Drug Metab. Toxicol. **4**(5): 619–627.

6 Dambisya YM, Wong CL, Chan K (1990) Effects of ephedrine and phenylpropanolamine on the antinociceptive effects of morphine and codeine in mice. Arch Int Pharmacodyn Ther. **308**: 5–12.

7 Dambisya YM, Chan K, Wong CL (1991) Role of adrenoceptors in the potentiation of opioid antinociceptin by ephedrine and phenylpropanolamine in mice. Psychopharmacol (Berl). **105**(4): 563–56725.

8 Dawson C, Ma D, Chow A, Maze M (2004) Dexmedetomidine enhances analgesic action of nitrous oxide: mechanisms of action. Anesthesiology. **100**: 894–904.

9 Guo TZ, Jiang JY, Buttermann AE, Maze M (1996) Dexmedetomidine injection into the locus ceruleus produces antinociception. Anesthesiology. **84**: 873–881.

10 Hall JE, Uhrich TD, Barney JA, Arain SR, Ebert TJ (2000) Sedative, amnestic and analgesic properties of small dose of dexmedetomidine infusions. Anest Analg. **90**: 699–705.

11 Hunter JC, Fontana DJ, Hedley LR, Jasper JR, Lewis R, Link RE, *et al.* (1997) Assessment of the role of α_2-adrenoceptor subtypes in the antinociceptive, sedative and hypothermic action of dexmedetomidine in transgenic mice Brit J Pharmacol. **122**: 1339–1344.

12 Ishiyama T, Kashimoto S, Oguchi T, Furuya A, Fukushima H, Kumazawa T (2006) Clonidine-ephedrine combination reduces pain on injection of propofol and blunts hemodynamic stress responses during the induction sequence. Journal of Clinical Anesthesia. **18**: 211–215.

13 Jacobs KM, Hirsch KA (2000) Psychiatric complications of mahuang. Psychosomatics. **41**(1): 58–62.

14 Kalso EA, Poyhia R, Rosenberg PH (1991) Spinal antinociception by dexmedetomidine, a highly selective alpha 2-adrenergic agonist. Pharmacol Toxicol. **68**(2): 140–143.

15 Kamibayashi T, Maze M (2000) Clinical uses of α_2-adrenergic agonists. Anesthesiology. **93**: 1345–1349.

16 Malmberg AB, Hedley LR, Jasper JR, Hunter JC, Basbaum AI (2001) Contribution of α_2-receptor subtypes to nerve injury-induced pain and its regulation by dexmedetomidine. Brit J Pharmacol. **132**: 1827–1836.

17 Nelson L, Lu J, Guo T, Saper C, Franks N, Maze M (2003) The [alpha]$_2$-Adrenoceptor agonist dexmedetomidine converges on an endogenous sleep-promoting pathway to exert its sedative effects. Anesthesiology. **98**(2): 428–436.

18 Pertovaara A, Kauppila T, Jyvasjarvi E, Kalso E (1991) Involvement of supraspinal and spinal segmental alpha-2-adrenergic mechanisms in the medetomidine-induced antinociception. Neuroscience. **44**: 705–714.

19 Pertovaara A (2006) Noradrenergic pain modulation. Prog Neurobiol. **80**(2): 53–83.

20 Philipp M, Brede M, Hein L (2002) Physiological significance of α_2-adrenergic receptor subtype diversity: one receptor is not enough. Am J Physiol - Reg I. **283**: 287–295.

21 Poree LR, Guo TZ, Kingery WS, Maze M (1998) The analgesic potency of dexmedetomidine is enhanced after nerve injury: a possible role for peripheral *α*-adrenoceptors. Anesth Analg. **87**: 941–948.

22 Quintin L, Bouilloc X, Butin E, Bayon MC, Brudon JR, Levron JC *et al.* (1996) Clonidine for major vascular surgery in hypertensive patients: a double-blind, controlled, randomized study Anesth. Analg. **83**: 687–695.

23 Sandler AN (1996) The role of clonidine and alpha2-agonists for postoperative analgesia. Can J Anaesth. **43**(12): 1195–1202.
24 Tekol Y, Tergan E, Esmaoğlu A (1994) Ephedrine enhances analgesic effect of morphine. Acta Anaesth Scand. **38**: 396–397.
25 Tekol Y, Koramaz Ş (1996) Ephedrine diminishes some side effects of morphine. Analgesia. **2**: 113–116.
26 Virtanen R, Savola JM, Saano V, Nyman L (1988) Characterization of the selectivity, specificity and potency of medetomidine as an alpha2-adrenoceptor agonist. Eur J Pharmacol. **150**(1–2): 9–14.
27 Wesolowska A, Young S, Dukat M (2004) MD-354 potentiates the antinociceptive effect of clonidine in the mouse tail-flick but not hot-plate assay. Eur J Pharmacol. **495**(2–3): 129–136.
28 Weischer ML (1976) Eine einfache Versuchsanordnung zur quantitativen Beurteilung von Motilität and Neugierverhalten bei Mäusen. Psychopharmacol. **50**: 275–279.
29 Zimmermann M (1983) Ethical guidelines for investigations of experimental pain in conscious animals. Pain. **16**: 109–110.