Title
Low Dose Alpha-2 Antagonist Paradoxically Enhances Rat Norepinephrine and Clonidine Analgesia

Journal
Anesthesia & Analgesia, 112(6)

ISSN
0003-2999

Authors
Milne, Brian
Sutak, Maaja
Cahill, Catherine M
et al.

Publication Date
2011-06-01

DOI
10.1213/ane.0b013e3182121bae

Low Dose Alpha-2 Antagonist Paradoxically Enhances Rat Norepinephrine and Clonidine Analgesia

Brian Milne, MD,*† Maaja Sutak,† Catherine M. Cahill, PhD,*† and Khem Jhamandas, PhD*†

Ultralow-dose opioid antagonists prolong opioid antinociception and block tolerance. In this study we determined whether low doses of the α-2 adrenergic receptor (A2-R) antagonist, atipamezole, similarly influenced A2-R-induced antinociception and tolerance. In rats, intrathecal norepinephrine (NE) or clonidine in combination with atipamezole was tested using tail-flick and paw pressure tests. Acute tolerance to NE was induced by serial injections. Low-dose atipamezole significantly prolonged NE and clonidine-induced antinociception. Coadministration of atipamezole with A2-R agonists also prevented loss of agonist potency in the acute tolerance model. This study demonstrates paradoxical effects of low-dose A2-R antagonists augmenting A2-R agonist-induced analgesia. (Anesth Analg 2011;112:1500–3)

Acute spinal norepinephrine (NE) and α-2 adrenergic receptor agonists, like opioids, produce potent analgesia, and their repeated administration induces tolerance.[1,2] Numerous reports, including clinical pilot studies, have demonstrated that ultralow doses of opioid receptor antagonists paradoxically augment spinal opioid-induced analgesia and block acute and chronic tolerance.[3–6] Considering the interaction between opioid and adrenergic receptors,[7,8] we examined whether ultralow doses of the selective α-2 antagonist, atipamezole, can similarly influence NE-induced spinal antinociception and tolerance. The specific objectives of the current investigation were to determine whether ultralow-dose atipamezole influenced spinal NE and clonidine-induced antinociception and the development of acute tolerance to repeated administration of spinal NE.

METHODS

Experiments were performed on male Sprague–Dawley rats (275 to 300 g) in accordance with the Guidelines of the Canadian Council on Animal Care after approval from the Queen's University Animal Care Committee. Animals were maintained on a normal light–dark cycle and allowed free access to food and water. Indwelling intrathecal catheter (7.5 cm, PE-10) implantation was performed under isoflurane anesthesia, as has been previously described.[9] Drugs under investigation were administered through the exteriorized portion of the catheter in a 10-μL volume and flushed with 10 μL of 0.9% saline. Control animals received equivalent injections of saline. All behavioral testing was performed without knowledge of the treatment between 0800 to 1400 hours.

The tail-flick test[10] evaluated the response to a brief thermal stimulus applied to the tail (5 cm from the end) with an analgesia meter.[11] Baseline latencies were set between 2 to 3 seconds and cutoff time at 10 seconds to prevent tissue damage. The paw pressure test evaluated responses to a brief mechanical nociceptive stimulus applied to the dorsal hindpaw by using an inverted air-filled syringe linked to a pressure gauge (baseline 80 to 100 mm Hg, cutoff time at 300 mm Hg).[12]

Acute Antinociception

A single ultralow dose of atipamezole (0.08 ng) (Farmos, Turku, Finland) was coinjected with NE (30 μg) (Sigma Chemical Co., St. Louis, MO) or clonidine (Sigma Chemical Co.) (13.3 μg + 0.008 or 0.0008 ng atipamezole) as a single intrathecal injection to determine its potential to alter α-2 adrenergic receptor-mediated antinociception.

Acute Tolerance

Tolerance to NE (30 μg) or in combination with atipamezole (0.8 or 0.008 ng) was induced by 3 serial injections (intrathecal) delivered at 90-minute intervals with animals tested over a 240-minute time period. Twenty-four hours after these serial injections, cumulative dose–response curves to the acute effects of NE were derived to obtain quantitative estimates of agonist potency (ED_{50} values). Tolerance was indicated by a loss of agonist potency (i.e., an increase in ED_{50} value).

All tail-flick and paw pressure values were converted to percentage of maximum possible effect (M.P.E.) (% MPE = 100 × (postdrug response − baseline response)/(cutoff response − baseline response)). Data are expressed as mean ± SEM for n = 4 to 11 per group. ED_{50} values were determined using nonlinear regression analysis. A 2-way repeated-measures analysis of variance (ANOVA) with time as a within-subject factor and treatment as a between-subjects factor was used to account for the repeated-measures design. A Time × Treatment interaction was included to test for differences in the longitudinal response patterns. Tukey post hoc tests were conducted where appropriate.

From the Departments of *Anesthesiology and Perioperative Medicine and †Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada.

Accepted for publication January 18, 2011.

Funding: Canadian Institutes of Health Research.

Conflict of Interest: See Disclosures at the end of the article.

Presented in part at the Canadian Anesthesiologists' Society Annual Meeting 2008.

Reprints will not be available from the authors.

Address correspondence to Catherine Cahill, PhD, Departments of Pharmacology & Toxicology and Anesthesiology & Perioperative Medicine, Queen's University, Kingston, Ontario, Canada K7L3N6. Address e-mail to cahillc@queensu.ca.

DOI: 10.1213/ANE.0b013e3182121bae

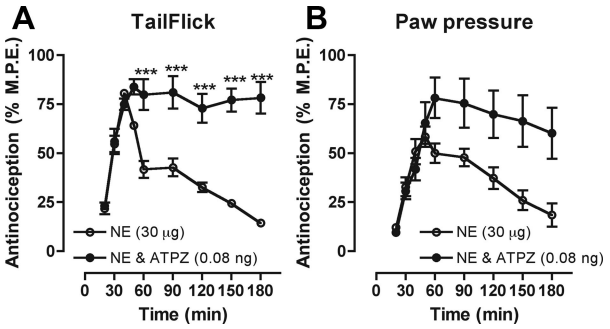

Figure 1. Low-dose atipamezole (ATPZ, 0.08 ng) augments acute spinal norepinephrine (NE) analgesia in the (A) tail-flick test and (B) paw pressure test. ATPZ or saline was combined with NE and delivered as a single intrathecal injection at time zero. A 2-way repeated-measures analysis of variance (ANOVA) revealed an effect of Treatment ($F_{(1104)}$ = 196.5, ***P < 0.001), Time ($F_{(10,104)}$ = 41.67,***P < 0.001), and a Time × Treatment interaction ($F_{(10,104)}$ = 13.74, ***P < 0.001) in the tail-flick test. A 2-way repeated-measures ANOVA of paw pressure data (B) revealed a significant effect of Treatment ($F_{(1104)}$ = 10.74, **P < 0.01), Time ($F_{(10,104)}$ = 12.55,***P < 0.001), but not a Time × Treatment interaction ($F_{(10,104)}$ = 1.319, P = 0.2302). Tukey post hoc analyses revealed which corresponding tail flick data points between the NE-only and ATPZ groups were significantly different (***P < 0.001). M.P.E. = maximum possible effect.

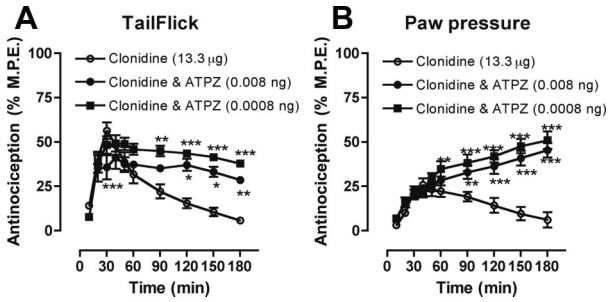

Figure 2. Low-dose atipamezole (ATPZ) augments clonidine analgesia in the tail-flick test (A) and paw pressure test (B). The time course of effects of ultralow-dose atipamezole (0.0008, 0.008 ng) on the acute antinociceptive action of clonidine (13.3 μg) is shown. A 2-way repeated-measures analysis of variance (ANOVA) of the tail-flick data revealed a significant effect of Treatment ($F_{(2160)}$ = 16.76, ***P < 0.001), Time ($F_{(9160)}$ = 33.71,***P < 0.001), and a Time × Treatment interaction ($F_{(18,160)}$ = 5.749, ***P < 0.001). A 2-way repeated-measures ANOVA on paw pressure data also revealed a significant effect of Treatment ($F_{(2160)}$ = 130.7, **P < 0.01), Time ($F_{(9160)}$ = 45.76,***P < 0.001), and a Time × Treatment interaction ($F_{(18,160)}$ = 16.91, ***P < 001). Tukey post hoc comparisons revealed which corresponding data points were significantly different between the clonidine-only and ATPZ treatment groups (*P < 0.05, **P < 0.01, ***P < 0.001). M.P.E. = maximum possible effect.

RESULTS

Intrathecal NE (30 μg) elicited a maximal antinociceptive effect that peaked 45 minutes (tail flick, paw pressure) after injection. Latencies returned to baseline levels 180 minutes after injection. Concomitant spinal administration with atipamezole (0.08 ng) prolonged the NE-induced antinociception in the tail-flick test (78% MPE at 180 minutes, Fig. 1A) and paw pressure test (60% at 180 minutes, Fig. 1B). This dose of atipamezole was far below that previously found to produce α-2 adrenergic receptor antagonism.[13] Ultralow-dose atipamezole similarly enhanced the antinociception produced by another α-2 adrenergic receptor agonist clonidine (13.3 μg, Fig. 2). Hence, concomitant spinal administration with atipamezole (0.008 or 0.0008 ng) prolonged the clonidine-induced antinociception in the tail-flick (Fig. 2A) and paw pressure tests (Fig. 2B).

Three successive intrathecal injections of NE (30 μg) 90 minutes apart resulted in a progressive loss of NE-induced antinociception illustrating acute tolerance. NE-induced antinociceptive effects at 240 minutes were reduced to nearly 35% of the initial response value at 30 minutes in the tail-flick test (Fig. 3A) and 22% of the initial response at 30 minutes in the paw pressure test (Fig. 3B). In addition, repeated intrathecal NE administration was associated with an approximately 2.5-fold increase in the ED_{50} value (Fig. 3C). In contrast, the combination of NE with a low dose of atipamezole prevented both the decline of the antinociceptive response (0.8 ng, Fig. 3) and the increase in ED_{50} values (0.8, 0.008 ng, Fig. 3C).

DISCUSSION

The results demonstrate that ultralow doses of an α-2 adrenergic receptor antagonist have the ability to augment agonist-mediated acute antinociception and to block the development of tolerance at doses far below those previously reported to have antagonistic effects at the α-2 adrenergic receptor.[13] The antitolerance effect of atipamezole is indicated by both the prevention of the progressive loss of the pharmacological response and decline in the agonist potency. The ultralow doses of atipamezole were selected by extrapolation from doses of the drug shown previously to influence morphine analgesia and tolerance.[13] Our goal was to investigate the lowest dose that produced consistent effects on analgesia without visible adverse effects on the mobility of animals. Scouting experiments were performed to establish doses that met this criteria and hence the difference between the doses used in the clonidine and NE experiments. We have previously demonstrated that low-dose α-2 receptor antagonists augment spinal morphine analgesia and inhibit the development of tolerance.[13] Although the mechanisms underlying this unusual effect of atipamezole remain unclear, it may be that it acts in this regard on the μ opioid-α-2 receptor complex[13,14] to prevent excitatory signaling by opiates, block the latent stimulatory response to the agonist, prevent hyperalgesia, and thus augment analgesia. Similar to the excitatory effects of opiates, first reported in electrophysiologic studies on the dorsal root ganglion neurons,[15,16] a low dose of clonidine can increase dorsal horn neuronal activity stimulated by nociceptive input,[17] and prolonged spinal administration of clonidine to rats causes thermal hypersensitivity, which reflects hyperalgesia.[18] A low dose of the antagonist may minimize or prevent the acute desensitization of receptor population–mediating analgesia or prevent the adaptive pronociceptive responses (such as *N*-methyl-D-aspartate receptor activation) that serve to limit adrenergic analgesia and contribute to the induction of analgesic tolerance.[18]

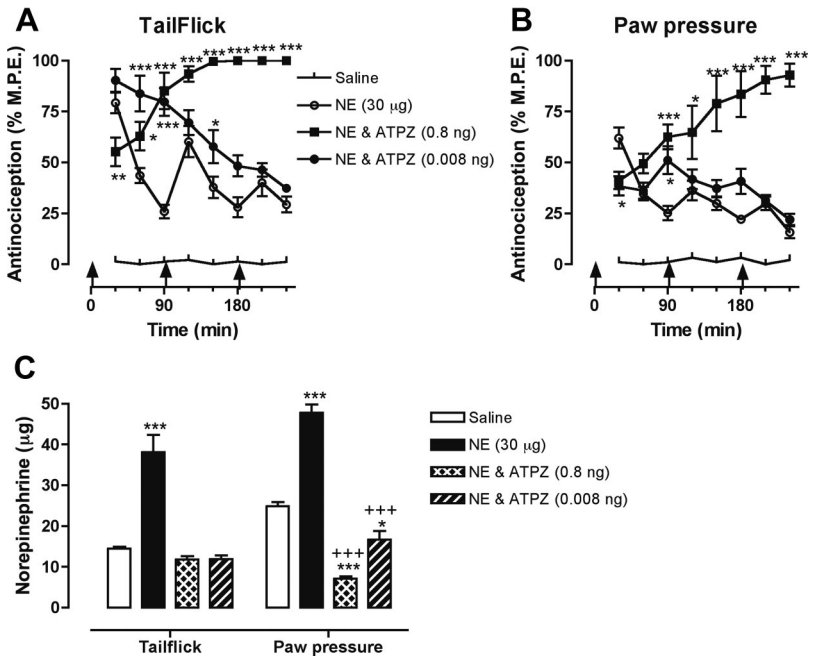

Figure 3. Effect of low-dose intrathecal atipamezole (ATPZ, 0.8, 0.008 ng) on the induction of acute tolerance to spinal norepinephrine (NE) in the (A) tail-flick test and (B) paw pressure test and cumulative dose–response curves 24 hours after acute tolerance testing (C). In panels A and B, NE (30 μg, intrathecal) was injected at 0, 90, and 180 minutes to induce acute tolerance, as is indicated by the arrows. Atipamezole was coadministered with NE as a single injection. Behavioral testing was performed at 30-minute intervals. A 2-way repeated-measures analysis of variance (ANOVA) of the tail-flick data revealed a significant effect of Treatment ($F_{(2,72)} = 46.00$, $^{***}P < 0.001$), Time ($F_{(8,72)} = 97.20$,$^{***}P < 0.001$), and a Time × Treatment interaction ($F_{(16,72)} = 30.26$, $^{***}P < 0.001$). Likewise, a 2-way repeated-measures ANOVA of the paw pressure data revealed a significant effect of Treatment ($F_{(2,72)} = 19.70$, $^{**}P < 0.01$), Time ($F_{(8,72)} = 19.70$,$^{***}P < 0.001$), and a Time ×Treatment interaction ($F_{(16,72)} = 17.20$, $^{***}P < 001$). Tukey post hoc comparisons revealed which corresponding data points between the NE-only and NE + ATPZ treatment groups were significantly different ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). In panel C, the NE ED_{50} values were obtained from cumulative dose–response curves to the antinociceptive action of NE 24 hours after the initial intrathecal drug treatment (panels A and B). One-way ANOVAs revealed significant differences between treatments both within the tail-flick paradigm ($F_{(3,14)} = 524.1$, $^{***}P < 0.001$) and within the paw pressure paradigm ($F_{(3,14)} = 1008$, $^{***}P < 0.001$). Tukey post hoc analysis were used to compare each treatment group with the saline controls ($^{*}P < 0.05$, $^{***}P < 0.001$) and with the NE-only-induced effects ($+++P < 0.001$). M.P.E. = maximum possible effect.

DISCLOSURES

Name: Brian Milne, MD.
Contribution: Study design and manuscript preparation.
Conflict of Interest: Brian Milne is one of the inventors on the patent filed by Parteq Innovations at Queens University based partially on this work.
Name: Khem Jhamandas, PhD.
Contribution: Study design and manuscript preparation.
Conflict of Interest: Khem Jhamandas is one of the inventors on the patent filed by Parteq Innovations at Queens University based partially on this work.
Name: Maaja Sutak.
Contribution: Conduct of study and data analysis.
Conflict of Interest: Maaja Sutak has declared no conflict of interest.
Name: Catherine Cahill, PhD.
Contribution: Manuscript preparation and data analysis.
Conflict of Interest: Catherine Cahill has declared no conflicts of interest.

REFERENCES

1. Milne B, Cervenko F, Jhamandas K, Loomis C, Sutak M. Analgesia and tolerance to intrathecal morphine and norepinephrine infusion via implanted mini-osmotic pumps in the rat. Pain 1985;22:165–72
2. Stevens CW, Monasky MS, Yaksh TL. Spinal infusion of opiate and alpha-2 agonists in rats: tolerance and cross tolerance studies. J Pharmacol Exp Ther 1988;244:63–70
3. Mattioli T, Milne B, Cahill C. Ultra-low dose naltrexone attenuates chronic morphine-induced gliosis in rats. Mol Pain 2010;6:22
4. Abul-Husn N, Sutak M, Milne B, Jhamandas K. Augmentation of spinal morphine analgesia and inhibition of tolerance by low doses of mu- and delta-opioid receptor antagonists. Br J Pharmacol 2007;151:877–87
5. Chindalore V, Craven R, Yu K, Butera P, Burns L, Friedman N. Adding ultralow-dose naltrexone to oxycodone enhances and prolongs analgesia: a randomized controlled trial of oxytrex. J Pain 2005;6:392–9
6. Powell KJ, Abul-Husn NS, Jhamandas A, Olmstead MC, Beninger RJ, Jhamandas K. Paradoxical effects of the opioid antagonist naltrexone on morphine analgesia, tolerance, and reward in rats. J Pharmacol Exp Ther 2002;300:588–96
7. Loomis CW, Cervenko FW, Jhamandas K, Sutak M, Milne B. Analgesia and autonomic function following intrathecal administration of morphine and norepinephrine to the rat. Can J Physiol Pharmacol 1985;63:656–62
8. Omote K, Kitahata LM, Collins JG, Nakatani K, Nakagawa I. Interaction between opiate subtype and alpha-2 adrenergic agonists in suppression of noxiously evoked activity of WDR neurons in the spinal dorsal horn. Anesthesiology 1991;74:737–43
9. Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976;17:1031–6
10. D'Amour FE, Smith DD. A method for determining loss of pain sensation. J Pharmacol Exp Ther 1941;72:74–9
11. Owen JA, Milne B, Jhamandas K, Nakatsu K. Assembly of an inexpensive tail flick analgesia meter. J Pharmacol Methods 1981;6:33–7

12. Sherman SE, Loomis CW, Milne B, Cervenko FW. Tolerance to intrathecal oxymetazoline-induced analgesia, with paradigm-dependent cross-tolerance to intrathecal morphine. J Pharmacol Exp Ther 1988;245:319–26
13. Milne B, Sutak M, Cahill CM, Jhamandas K. Low doses of α2-adrenoceptor antagonists augment spinal morphine analgesia and inhibit development of acute and chronic tolerance. Br J Pharmacol 2008;155:1264–78
14. Tan M, Walwyn WM, Evans CJ, Xie C. p38 MAPK and β-arrestin 2 mediate functional interactions between endogenous μ-opioid and α2A-adrenergic receptors in neurons. J Biol Chem 2009;284:6270–81
15. Crain SM, Shen KF. Ultra-low concentrations of naloxone selectively antagonize excitatory effects of morphine on sensory neurons, thereby increasing its anti-nociceptive potency and attenuating tolerance/dependence during chronic cotreatment. Proc Natl Acad Sci USA 1995;92:10540–4
16. Crain SM, Shen KF. Antagonists of excitatory opioid receptor functions enhance morphine's analgesic potency and attenuate opioid tolerance/dependence liability. Pain 2000;84:121–31
17. Sullivan AF, Dashwood MR, Dickenson AH. Alpha 2-adrenoceptor modulation of nociception in rat spinal cord: location, effects and interactions with morphine. Eur J Pharmacol 1987;138:169–77
18. Quartilho A, Mata HP, Ibrahim MM, Vanderah TW, Ossipov MH, Lai J, Porreca F, Malan TP Jr. Production of paradoxical sensory hypersensitivity by alpha 2-adrenoreceptor agonists. Anesthesiology 2004;100:1538–44