■ LABORATORY INVESTIGATIONS

Ketamine, an N-methyl-D-aspartate Receptor Antagonist, Inhibits the Reflex Responses to Distension of the Rat Urinary Bladder

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Background: Ketamine is analgesic in experimental and clinical studies of inflammatory, neuropathic, and postoperative pain. Its role in the treatment of visceral pain is less known. The authors investigated the effect and site of action of ketamine on reflex responses evoked by urinary bladder distension (UBD). The effects of other clinically available N-methyl-D-aspartate (NMDA) receptor antagonists on these responses were also studied.

Methods: The effect of intravenous ketamine (1, 3, and 10 mg/kg), dextromethorphan (5 mg/kg), and memantine (16 mg/kg) on mean arterial pressure changes (ΔMAP) and abdominal electromyographic activity (EMG) evoked by UBD was measured in anesthetized rats. Ketamine was also administered intravesically and intrathecally and its effect on ΔMAP and EMG responses to UBD recorded. The effect of pretreatment with intravenous ketamine on these responses was also assessed.

Results: The ΔMAP and EMG responses to UBD were reduced in a dose-dependent fashion by ketamine. Memantine and dextromethorphan also inhibited these responses. Ketamine administered intrathecally produced marked inhibition of ΔMAP and EMG responses to UBD. Pretreatment with ketamine only transiently reduced the vigor of responses to UBD.

Conclusions: Ketamine inhibited, in a dose-dependent fashion, the ΔMAP and EMG responses to UBD, an effect likely caused by actions within the spinal cord. Similar inhibition observed with systemic dextromethorphan and memantine treatments suggests that the analgesic effect of ketamine is caused by antagonism of the NMDA receptor. Pretreatment with ketamine did not have a preventive effect in this model of bladder nociception.

THE role of the *N*-methyl-D-aspartate (NMDA) receptor in the mechanisms of central sensitization has been extensively described in models of somatic pain[1,2]; however, a different role of NMDA receptors in the spinal processing of nociceptive inputs originating in the viscera has been proposed.[3,4] Apart from the role of these receptors in the mechanisms of visceral hyperalgesia and central

sensitization,[5–8] NMDA receptors have also been demonstrated to have effects on the processing of acute nociception from visceral structures.[4,9,10]

There is extensive literature related to the analgesic properties of ketamine, an NMDA receptor antagonist, in experimental pain models, as well as in clinical trials related to somatic pain and nociception.[1] However, few studies have examined the analgesic effect of ketamine in models of visceral pain. Those studies have examined responses to distension of the colon[9,10] or ureter[4] and compared them with the effect of ketamine on responses to somatic nociceptive stimuli.

To the best of our knowledge, no experimental studies have been performed related to the effect of ketamine on pain originating in the urinary bladder. Likewise, the effect of ketamine on the prevention of visceral hyperalgesia has not been investigated, but other experimental NMDA receptor antagonists, which are not available clinically, have been tested with this purpose.[7]

We recently characterized cardiovascular and visceromotor responses to urinary bladder distension (UBD) in the halothane-anesthetized rat.[11,12] These responses are reliable, reproducible, and inhibited in a dose-dependent fashion by analgesics such as morphine, suggesting that they are useful end points to assess urinary bladder nociception. We have also observed that reflex responses become more vigorous after the presentation of repeated UBD, reflecting a phenomenon of sensitization.[11,12] The mechanism of this observation remains unknown.

The current study sought to characterize the effect of ketamine in a model of visceral nociception originating in the urinary bladder. This was investigated using the mean arterial pressure changes (ΔMAP) and electromyographic activity (EMG) responses to the mechanical distension of the bladder as nociceptive end points. The effect of two other clinically available NMDA receptor antagonists, dextromethorphan and memantine, on these responses to UBD allowed for a pharmacologic comparison. A comparison of the effects on reflex responses to UBD of the same dose of ketamine administered by different routes (*i.e.*, intravenously *vs.* intravesically *vs.* intrathecally) was performed in an attempt to describe possible sites of action of the drug. Finally, the effect of ketamine administered as a pretreatment on the increasing vigor of responses caused by repeated UBD was investigated.

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Materials and Methods

General

These studies were approved by our institutional review board for nonhuman studies. Female Sprague-Dawley rats were anesthetized with halothane (2–5%) delivered by mask. Jugular venous, carotid arterial, and tracheal cannulae were placed, and two wire electrodes were sutured to the oblique abdominal musculature just above the inguinal ligament allowing for differential amplification of their myoelectric activity (EMG activity). In eight rats, intrathecal catheters were inserted through a small opening in the atlanto-occipital membrane and passed 4.5 cm caudally in the intrathecal space. After completing the surgical preparation, the halothane anesthesia was reduced until flexion reflex responses could be evoked by pinch of the foot but without spontaneous escape behaviors (typically 0.5–0.7% halothane). Rats without the presence of a vigorous flexion reflex response to pinch of the foot before pharmacologic manipulations were excluded from the study. The rats were artificially ventilated using a volume-cycled respirator. Rats were not restrained in any fashion. Body temperature was maintained using a heating pad and overhead radiant lights.

Cardiovascular and Visceromotor Responses

Cardiovascular parameters were measured continuously *via* the carotid cannula and a low volume transducer. Cardiovascular responses were defined as the peak change in mean arterial pressure (ΔMAP, mmHg) during UBD compared with the average MAP during a 10-s prestimulus period. The myoelectric activity was differentially amplified using standard methodology (Grass P511 AC Amplifier, 100 X amp., Astro-Med, Inc., West Warwick, RI) and converted to digital information using a Micro 1401 processor and associated Spike-2 software (Cambridge Electronic Design, Cambridge, UK) and a previously described analysis routine.[12] The signal was quantified as the number of episodes of nonrectified electromyographic activity exceeding a preset "threshold" voltage limit. This threshold was individually adjusted above the baseline level of activity of the abdominal musculature before the administration of UBD. The same threshold level was maintained throughout the course of a given experiment. The number of episodes above the threshold level was determined for 10 s before UBD and during the 20 s of UBD, the former serving as a measure of spontaneous activity and the latter of total activity during UBD. Subtraction of spontaneous activity from total activity during UBD allowed for the calculation of UBD-evoked activity, which was defined as the visceromotor response to UBD. Because quantified EMG responses of the abdominal musculature to the same stimulus vary in maximal response in relation to the site where the recording wires were placed, EMG activity for

each experiment was normalized as a percentage of that produced by baseline UBD responses (60 mmHg, 20 s) for most experiments.

Urinary Bladder Distension

In all of the animals studied, a 22-gauge Teflon angiocatheter was placed into the urinary bladder *via* the urethra and held in place by a tight suture around the distal urethral orifice. UBD was administered as phasic stimuli (rapid onset, rapid offset) using compressed air and a previously described distension control device.[13] Standardized UBD consisted of 60 mmHg, 20 s phasic distension stimuli at 4 min intervals.[11,12] Stimulus response functions (SRFs) were constructed for graded data evoked by graded, phasic UBD (20–80 mmHg, 20 s) given in sequence.

Drug Protocol

All pharmacologic manipulations were performed as single boluses. A fresh solution of ketamine (1, 3, and 10 mg/kg), dextromethorphan (5 mg/kg), or memantine (16 mg/kg) (Sigma Chemical Company, St. Louis, MO) was dissolved in saline for each experiment. The doses of ketamine were chosen based on previous studies of the effect of the drug on reflex responses to colorectal and ureteral distension.[10,4] Doses for dextromethorphan and memantine were those found to be effective in studies by Dickenson *et al.*[14] and Olivar and Laird[4] respectively. When drugs were administered intravenously, 1 ml/kg of volume solution was used and the same volume of saline administered intravenously was used as control. Using the solution described previously, 3 mg/kg of ketamine in 1 ml/kg of volume was also administered intravesically by the urethral catheter using a 1 ml syringe and maintained inside the urinary bladder for 15 min, after which the bladders were allowed to drain spontaneously. Ketamine (3 mg/kg) was also administered by intrathecal catheters in a 10 μl volume, followed by a flush of 10 μl of normal saline. The same volume of saline (total 20 μl) administered intrathecally was used as control.

To examine the bladder sensitization process we have noted before,[11,12] at the beginning of most experiments 1 ml/kg of normal saline was injected intravenously and ten 60 mmHg, 20 s phasic UBD stimuli at 4 min intervals were performed. This constituted the control group with which the ketamine pretreatment group was compared (this is described later in the article). With the exception of the ketamine pretreatment group, pharmacologic manipulations were performed after the ten distensions described previously were performed and three baseline responses to UBD (60 mmHg, 20 s) followed by one trial of graded responses to UBD (20–80 mmHg, 60 s) were obtained. Drugs were injected over 1 min, and ΔMAP and EMG responses to UBD (60 mmHg, 20 s, every 4 min) followed for 40 min after injections. To

Table 1. Experimental Baseline Conditions

	MAP (mmHg)	ΔMAP (mmHg)	EMG (cts)
Saline (IV) (n = 6)	120 ± 4	17 ± 2	323 ± 86
Ketamine 1 mg/kg (IV) (n = 4)	114 ± 7	13 ± 3	256 ± 96
Ketamine 3 mg/kg (n = 6)	113 ± 4	17 ± 2	141 ± 46
Ketamine 10 mg/kg (n = 5)	124 ± 4	19 ± 3	187 ± 83
Memantine (n = 6)	111 ± 2	21 ± 4	219 ± 68
Dextromethorphan (n = 5)	115 ± 8	17 ± 5	300 ± 80
Saline (it) (n = 3)	118 ± 5	14 ± 2	283 ± 107
Ketamine 3 mg/kg (it) (n = 5)	123 ± 4	15 ± 4	455 ± 65*
Ketamine 3 mg/kg (ives) (n = 3)	120 ± 7	19 ± 9	271 ± 142

All data is presented as mean ± SEM. ΔMAP and EMG responses to urinary bladder distension are the average of the three baseline responses.

* $P < 0.05$ unpaired t test with Bonferroni correction, compared with the group treated with 1 mg/kg of intravenous ketamine.

MAP = mean arterial pressure (mmHg); EMG = electromyographic activity produced by UBD (60 mmHg, 20 s) (counts); ΔMAP = change in arterial blood produced by UBD (60 mmHg, 20 s) (mmHg); IV = intravenous.

study SRFs without interfering with treatments' time frame descriptions, second single boluses of drugs or saline were administered when 80% of the baseline responses to UBD were recovered. ΔMAP and EMG responses to graded UBD (20–80 mmHg) were obtained before, and 12 min after, second injections.

In eight rats, ketamine (3 mg/kg) was administered intravenously before any UBD was performed. Forty-four rats were treated with 1 ml/kg of normal saline intravenously, following the same methodology. The effect of ketamine or saline in the process of increasing vigor of ΔMAP and EMG responses produced by the application of 10 UBD (60 mmHg, 20 s every 4 min) was evaluated. Quantification of EMG activity was performed using the same predetermined threshold value for all the animals and data expressed as the number of events above such threshold. For this part of the study, raw data are presented, since baseline responses are obtained before drug injections and no normalization procedure was deemed appropriate.

Statistics

Statistics are presented as the mean ± SEM. Data are presented as percentage of the average of three baseline responses for both ΔMAP and EMG responses to UBD, unless otherwise stated. Unpaired t test with Bonferroni correction for multiple comparisons was used to compare groups in baseline conditions. Comparisons with control responses were done using unpaired t test and two-way repeated measures analysis of variance (ANOVA) followed by *post hoc* analysis with Tukey–Kramer procedure. The statistical program used for analysis was GB-STAT (Dynamic Microsystems, Inc., Silver Springs, MD). Statistical significance was defined as P values ≤ 0.05.

Results

Mean arterial blood pressure (MAP) in resting conditions as well as ΔMAP in response to UBD (60 mmHg,

20 s) before pharmacologic manipulations did not show any statistically significant differences between the study groups ($P > 0.05$, unpaired t test with Bonferroni correction for multiple comparisons) (table 1). Likewise, no statistically significant differences between groups was observed in the EMG response to UBD before drug injections, with the exception of the intrathecal ketamine group, in which responses were more vigorous than those observed in the 3 mg/kg intravenous ketamine group (455 ± 65 *vs.* 141 ± 46, $P < 0.05$, unpaired t test) (table 1). There was some variability between subjects on the EMG response to UBD in relation to the site where the recording wires were placed, and minor differences in the surgical preparation related to the insertion of the intrathecal catheters could explain such difference.[15]

Effect of Intravenous Ketamine

A typical example of the ΔMAP and EMG responses to UBD in baseline condition and after intravenous ket-
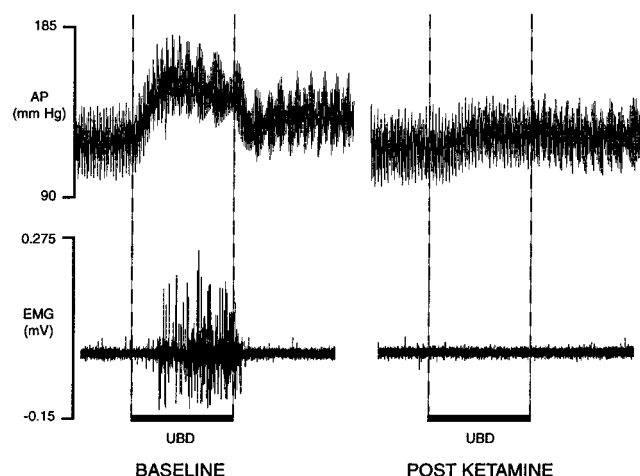
Fig. 1. Typical example of change in mean arterial blood pressure (ΔMAP) and electromyographic (EMG) activity evoked by urinary bladder distension (UBD, 60 mmHg, 20 s) in baseline conditions (*left*) and after the intravenous administration of 10 mg/kg of ketamine (*right*). AP = arterial pressure (mmHg).
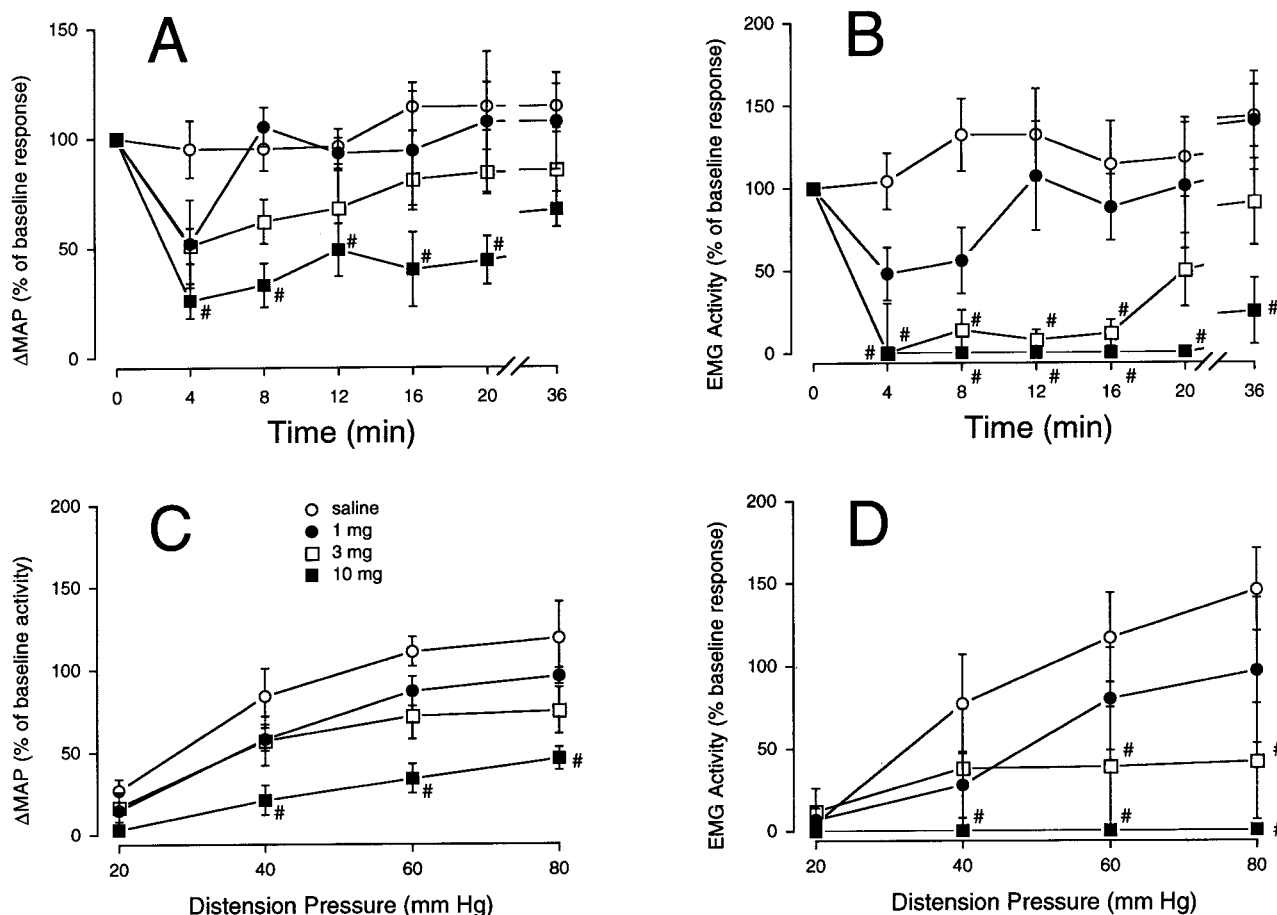
Fig. 2. Effect of ketamine administered intravenously on reflex responses to urinary bladder distension (UBD). Time dependent effects on: (*A*) change in mean arterial blood pressure (ΔMAP) and, (*B*) abdominal electromyographic (EMG) activity evoked by UBD (60 mmHg, 20 s, every 4 min) after treatment with single boluses of ketamine 1 (n = 4), 3 (n = 6), 10 mg/kg (n = 5) or normal saline (n = 6). (*C*) and (*D*): effect of same doses of ketamine or saline on ΔMAP and EMG responses to graded UBD respectively. Data are presented as percentage of baseline responses and expressed as mean ± SEM. #*P* < 0.01, two-way repeated measures analysis of variance test followed by *post hoc* analysis with Tukey–Kramer procedure.

amine (10 mg/kg) is shown in figure 1. Effect of repeated measures on response variability has been previously reported elsewhere.[11,12]

Time Dependent Effects. Single boluses of 1 mg/kg (n = 4), 3 mg/kg (n = 6), 10 mg/kg (n = 5), or the same volume of saline (1 ml/kg, n = 6) were administered over 1 min. A slight, transient reduction in the MAP from resting conditions was observed after the injection of ketamine. Similar change was observed after intravenous injection of saline. By the time the first UBD was performed, MAP had returned to the previous baseline value in all the experiments.

Ketamine produced a dose-dependent inhibition of the ΔMAP and EMG responses to UBD (60 mmHg, 20 s). Four minutes after the injection of 1, 3, and 10 mg/kg of ketamine, the ΔMAP response to UBD (60 mmHg, 20 s) decreased to 52 ± 20%, 51 ± 10%, and 26 ± 8% of the baseline responses, respectively. Results of 3 and 10 mg/kg reached statistical significance when they were compared with the effect produced by saline at the same time point (*P* < 0.01 and *P* < 0.0001 respectively,

unpaired *t* test). The effect of 1 and 3 mg/kg on ΔMAP responses to UBD was brief and two-way repeated measures ANOVA tests followed by *post hoc* analysis with Tukey–Kramer procedure did not show statistically significant difference at any time point. A time course of the effect of ketamine on ΔMAP responses to UBD and statically significant differences observed with 10 mg/kg of ketamine (two-way repeated measures ANOVA test followed by *post hoc* analysis with Tukey–Kramer procedure) are presented in figure 2A.

Ketamine showed a greater and more prolonged inhibition of the EMG responses to UBD than that observed for the ΔMAP responses. 1 mg/kg of ketamine reduced the EMG baseline response to UBD (60 mmHg, 20 s) to 48 ± 16% after 4 min of the injection (*P* < 0.05, unpaired *t* test) and 3 or 10 mg/kg abolished the EMG response at the same time point. The effect of ketamine was also more prolonged on the EMG response to UBD than the ΔMAP response, as is shown in figure 2A and B. Likewise, the ketamine inhibition of the EMG response to UBD (60 mmHg, 20 s) was more prolonged with

higher doses (fig. 2B). Two-way repeated measure ANOVA test followed by *post hoc* analysis with Tukey–Kramer procedure showed statistically significant differences after 3 and 10 mg/kg of ketamine (fig 2B).

Effect on Stimulus-response Functions. Figures 2C and 2D demonstrate the effect of ketamine on the stimulus response functions relating to ΔMAP and EMG responses to graded UBD. A dose-dependent reduction of the slopes of these functions for ΔMAP is observed after single boluses of intravenous ketamine. Two-way repeated measures ANOVA test followed by *post hoc* analysis with Tukey–Kramer procedure showed statistically significant differences in comparison with saline effects on the ΔMAP produced by 40, 60, and 80 mmHg ($P <$ 0.01) only with the highest dose of ketamine (10 mg/kg). A greater effect of ketamine on the stimulus response functions for EMG response to graded UBD was observed after the administration of ketamine, with a complete abolition of these responses at the highest dose of the drug tested (Fig. 2D). Two-way repeated measures ANOVA test followed by *post hoc* analysis with Tukey–Kramer procedure showed statistically significant differences comparing ketamine with saline effects on the EMG responses produced by 40 ($P < 0.01$ at 10 mg/kg), 60 ($P < 0.01$ at 3 and 10 mg/kg), and 80 mmHg of UBD pressure ($P < 0.01$ at 3 and 10 mg/kg).

Using two-way repeated measures ANOVA test followed by *post hoc* analysis with Tukey–Kramer procedure, no statistically significant differences in ΔMAP and EMG responses to UBD were observed with the lowest dose of ketamine tested (1 mg/kg) when compared with the saline group.

Effect of other NMDA Receptor Antagonists

The effect of two other NMDA receptor antagonists was also investigated. Intravenous memantine (16 mg/kg, n = 6) and dextromethorphan (5 mg/kg, n = 5) were administered and their effect on the ΔMAP and EMG to UBD responses was studied after the same protocol described for ketamine. Memantine and dextromethorphan produced a transient reduction in the MAP from baseline conditions during injection. The MAP returned to the baseline levels before the presentation of the first UBD postinjection in most of the experiments. Four minutes after the injection of memantine, the ΔMAP response to UBD (60 mmHg, 20 s) decreased to 35 ± 15% of the baseline response. This effect was statistically significant when compared with the effect produced by saline at the same time point ($P = 0.01$, unpaired *t* test). The time course of the effect of memantine on the ΔMAP response to UBD (60 mmHg, 20 s every 4 min) is shown in Figure 3A. As was observed after ketamine injections, memantine showed a greater and more prolonged inhibition of the EMG responses to UBD than that observed for the ΔMAP responses. Memantine reduced the EMG responses to UBD (60 mmHg, 20 s) to 10 ± 11% after

4 min of the injection, reaching a statistically significant difference when compared with saline at the same time point ($P < 0.001$, unpaired *t* test). The effect of memantine was also more prolonged on the EMG response to UBD, as is shown in figure 3B.

Two-way repeated measures ANOVA test followed by *post hoc* analysis with Tukey–Kramer procedure showed statistically significant differences comparing memantine with saline effects in most of the time points for ΔMAP and EMG responses to UBD (fig. 3A and B).

Dextromethorphan had a different time course than the other two drugs studied, with a delay in its effect on responses to UBD. A reduction of the ΔMAP responses to UBD, in comparison with saline, was observed 20 min after injection of the drug with a maximal effect 28 min after injection (61 ± 13% of the baseline response). No recovery of the ΔMAP response to UBD was observed even 40 min after injection (fig. 3A). A shorter delay in dextromethorphan effect was observed on the EMG response to UBD, with a reduction in this response 12 min after injection of the drug (64 ± 13% of the baseline response). Maximal effect was observed 40 min after the injection (to 45 ± 22% of the baseline response, $P <$ 0.05, unpaired *t* test). Figures 3A and B show time course of the effect of dextromethorphan on the ΔMAP and EMG responses to UBD (60 mmHg, 20 s every 4 min) and statistically significant differences in comparison with saline effects using two-way repeated measures ANOVA test followed by *post hoc* analysis with Tukey–Kramer procedure.

Figures 3C and 3D illustrate the stimulus response functions relating ΔMAP and EMG activity responses to graded UBD after a repeat intravenous dose of memantine and the single bolus of dextromethorphan. A reduction of the slopes of these functions for ΔMAP and EMG responses to graded UBD (20–80 mmHg) is observed with both drugs. The changes in the stimulus-response functions produced by both memantine and dextromethorphan are qualitatively similar to that produced by ketamine. Figures 3C and 3D show statistically significant differences in stimulus-response functions between pharmacologic treatment groups and the control group, using two-way repeated measures ANOVA test followed by *post hoc* analysis with Tukey–Kramer procedure.

Site of Action of Ketamine

To investigate the possible sites of action of ketamine, the drug was also administered intrathecally (n = 5) and intravesically (n = 3), using the same dose used when administered intravenously. A transient but more profound reduction in the MAP from baseline conditions was observed after the intrathecal injection of ketamine, in comparison with the reduction observed with the same dose administered intravenously. The MAP returned to baseline values between the third and fourth UBD postinjection.
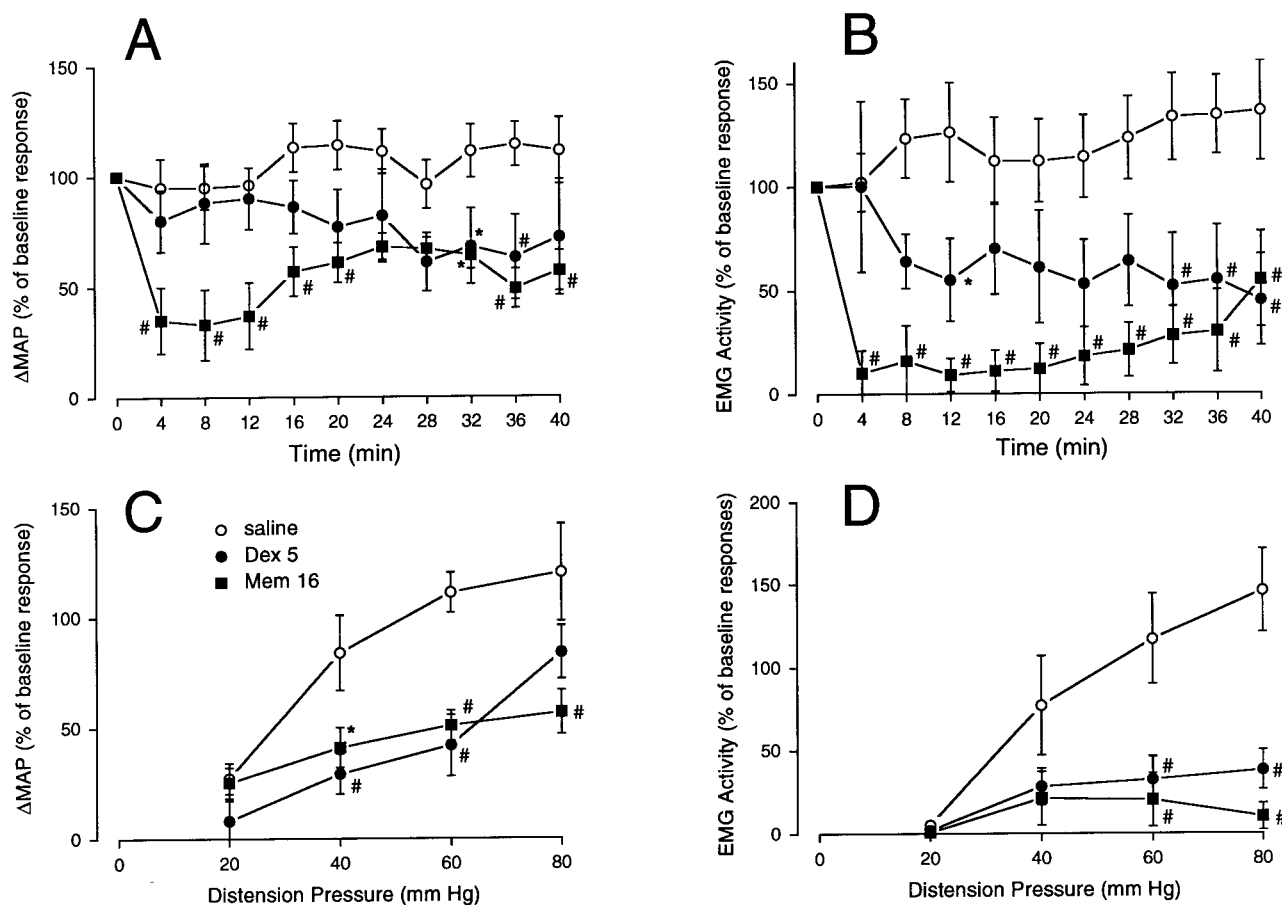
Fig. 3. Effects of intravenous dextromethorphan and memantine on reflex responses to urinary bladder distension (UBD). Time dependent effects on: (A) change in mean arterial blood pressure (ΔMAP) and (B) abdominal electromyographic (EMG) activity evoked by UBD (60 mmHg, 20 s, every 4 min) after treatment with 5 mg/kg of dextromethorphan (n = 5), 16 mg/kg of memantine (n = 6), or normal saline (n = 6; same animals as Fig. 2). In (C) and (D): effect of same doses of dextromethorphan, memantine, or normal saline on ΔMAP and EMG responses to graded UBD respectively. Data are presented as percentage of baseline responses and expressed as mean ± SEM. *P < 0.05 and #P < 0.01, two-way repeated measures analysis of variance test followed by *post hoc* analysis with Tukey–Kramer procedure.

Ketamine, when administered intrathecally, produced a greater and more prolonged inhibition of the ΔMAP to UBD (60 mmHg, 20 s) than that observed after the same dose of intravenous ketamine. Four minutes after the intrathecal injection of 3 mg/kg of ketamine, the ΔMAP response to UBD (60 mmHg, 20 s) decreased to 20 ± 14% of the baseline response ($P < 0.05$, unpaired t test). EMG response to UBD was abolished at the same time point, similar to the effect of intravenous ketamine, but this inhibition was more prolonged. A time course of the effect of intrathecal ketamine and saline on ΔMAP and EMG responses to UBD is presented in figure 4. As was observed with the intravenous injection of ketamine, intrathecal ketamine produced a greater inhibition of EMG responses than ΔMAP responses to UBD (60 mmHg, 20 s) (fig. 4A and B). Figure 4 shows statistically significant differences between the group treated with intrathecal ketamine and the control group, using two-way repeated measures ANOVA test followed by *post hoc* analysis with Tukey–Kramer procedure.

The intravesical administration of 3 mg/kg of ketamine

in 1 ml/kg of volume did not produce quantitatively significant changes in the ΔMAP and EMG responses to UBD (60 mmHg, 20 s). Four minutes after ketamine was released from the bladder, ΔMAP and EMG responses were 116 ± 42% and 94 ± 17% of the baseline responses, respectively.

Effect of Ketamine Pretreatment on ΔMAP and EMG Responses to Repeated UBD

The effect of intravenous ketamine (3 mg/kg) administered before the presentation of any UBD (pretreatment) on the ΔMAP and EMG responses to UBD (60 mmHg, 20 s) was studied in 8 rats and compared with the control group treated in a similar fashion with 1 ml/kg of normal saline (n = 44 for ΔMAP responses, n = 31 for EMG responses to UBD; fig. 5). An increase on vigor of both responses is observed after the application of 10 UBD (60 mmHg, 20 s every 4 min). No difference in the ΔMAP responses was observed after pretreatment with intravenous ketamine when compared with control rats during the whole series of 10 UBD (60 mmHg, 20 s) (fig. 5A). In
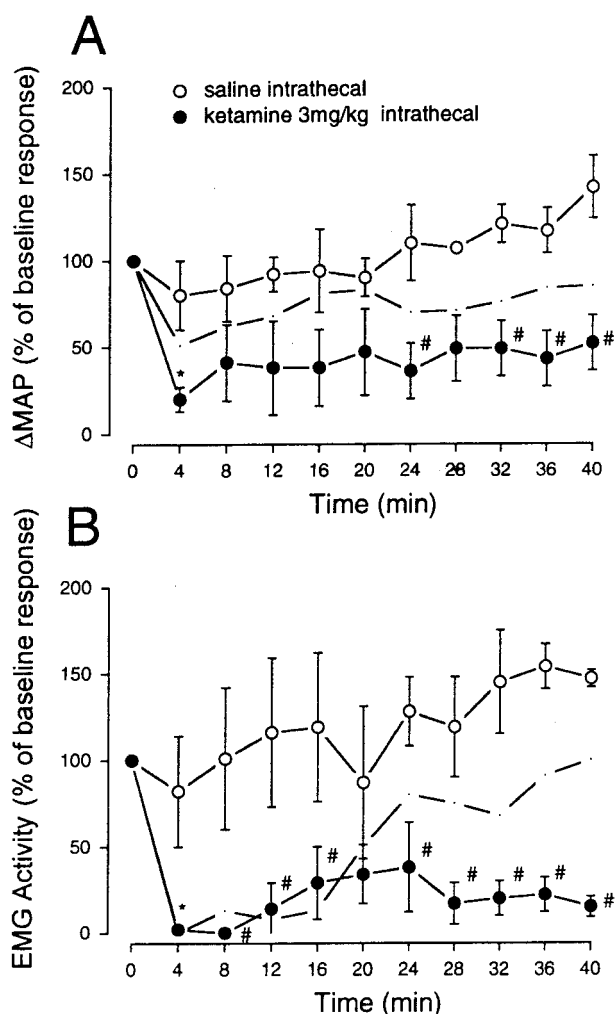
Fig. 4. Effect of intrathecal administration of ketamine on reflex responses to urinary bladder distension (UBD). Time dependent effects on change in mean arterial blood pressure (ΔMAP) (*A*) and abdominal electromyographic (EMG) activity (*B*) evoked by UBD (60 mmHg, 20 s, every 4 min) after treatment with a single bolus of intrathecal ketamine 3 mg/kg (n = 5) or the equivalent volume of normal saline (n = 3). The broken line with dots shows mean values of ΔMAP and EMG responses to UBD after the intravenous administration of a same dose of ketamine (fig. 2). Data are presented as percentage of baseline responses and expressed as mean ± SEM. * $P < 0.05$ and # $P < 0.01$, two-way repeated measures analysis of variance test followed by *post hoc* analysis with Tukey–Kramer procedure.

contrast, quantitative differences in the EMG responses of rats pretreated with ketamine were observed during the first seven UBD, with no EMG response to UBD during the first four UBD trials (fig. 5A).

Discussion

To the best of our knowledge, this is the first report of a characterization of the analgesic effects of ketamine in a model of urinary bladder nociception. Ketamine administered intravenously produced a dose-dependent inhibition of the ΔMAP and EMG responses to UBD. The
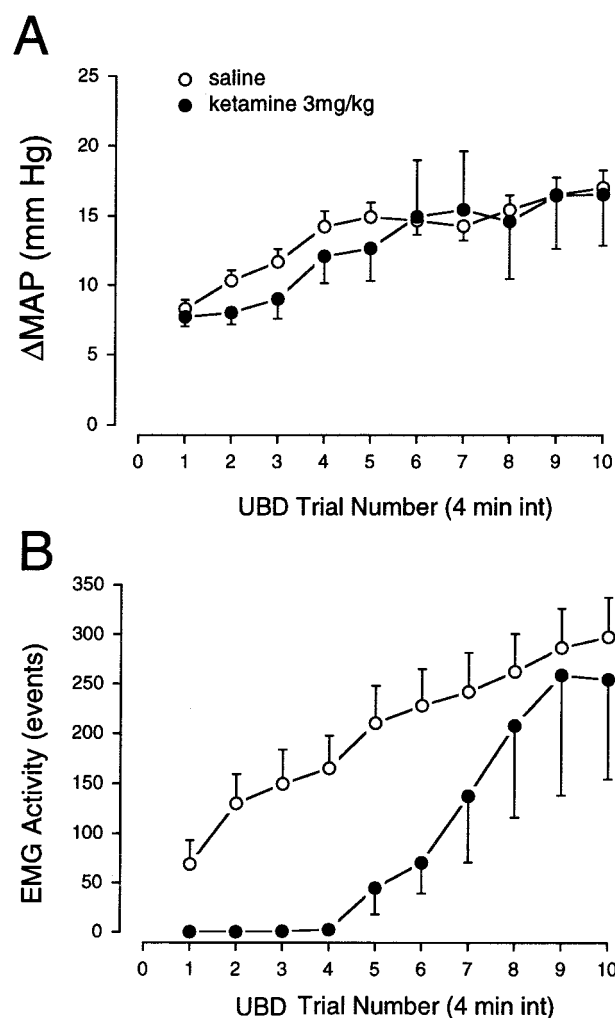

Fig. 5. Effect of repeat bladder distension at 60 mmHg for 20 s every 4 min after pretreatment with intravenous ketamine (3 mg/kg) or normal saline on reflex responses to urinary bladder distension (UBD). (*A*) Change in mean arterial blood pressure (ΔMAP) after saline (n = 44) and ketamine (n = 8); (*B*) abdominal electromyographic activity evoked by repeated UBD after same pretreatments (saline, n = 31; ketamine, n = 8). Data are mean ± SEM.

inhibitory effect of ketamine on these responses occurred at those UBD intensities that are painful to humans. Distension pressures in the range of 20 to 40 mmHg produce discomfort in humans during experimental urinary bladder distension.[16,17]

The measurement of reflex responses (*i.e.*, ΔMAP and EMG) to a visceral nociceptive stimulus has been extensively used in models of visceral pain.[18,19] These responses, characterized initially by Sherrington, consist of strong autonomic and motor reflex responses such as increases in arterial blood pressure, heart rate, and abdominal contractions in response to a visceral nociceptive stimulus.[20] Using the reflex responses (ΔMAP and EMG) to UBD as nociceptive end points, the effect of analgesic drugs such as morphine, fentanyl, and lidocaine have been investigated.[11,12] Altogether, these data

support the use of this preparation as a model of urinary bladder nociception and reinforce the concept that the inhibitory effects of ketamine on the ΔMAP and EMG responses to UBD are antinociceptive in nature. These results are similar to those observed in other models of visceral pain. Olivar and Laird found a dose-dependent inhibition of cardiovascular responses to ureteral distension by the same doses of systemic ketamine[4] and Alam *et al.* observed similar results using the visceromotor responses to colorectal distension.[10] Interesting enough, in both studies ketamine showed a weak or insignificant effect on responses to somatic nociceptive stimuli. Based on this finding the authors postulated a differing role for NMDA receptors in visceral and somatic nociception.

We used two different end points in our evaluation of the antinociceptive effects of ketamine. The fact that both were affected in a similar qualitative fashion by systemic ketamine supports the argument that the inhibition observed is related to an antinociceptive effect and not to a nonspecific action of the drug. Moreover, if we quantitatively compare the effect of ketamine on both responses, we find a greater and more prolonged effect of the drug on the EMG responses to UBD. This finding may reflect a greater sensitivity of these responses to the analgesic properties of ketamine, although part of such greater effect may be caused by a NMDA receptor antagonist motor effect with the highest dose. This motor effect is unlikely to be present with lower doses of ketamine.

Ketamine interacts with many receptors and circuits related to nociceptive neurotransmission, which may be related to its analgesic effects.[21–24] We found an inhibition of the ΔMAP and EMG responses to UBD (60 mmHg, 20 s) with a reduction of the slope of the stimulus response functions to graded UBD (20–80 mmHg) after the intravenous injection of NMDA receptor antagonists memantine and dextromethorphan. Interestingly, both drugs produced a greater and more prolonged inhibition of the EMG responses to UBD in comparison with their effect on ΔMAP responses, sharing this pattern with systemic ketamine. Olivar and Laird found a similar inhibition of the ΔMAP response to the distension of the ureter with 16 and 32 mg/kg of memantine administered in cumulative doses.[4] To our knowledge, no studies of the effect of dextromethrophan in models of visceral pain have been performed. The quantitative and qualitative similarity between the effect observed with these NMDA receptor antagonists and ketamine supports the argument that the effect observed with the latter on ΔMAP and EMG responses to UBD is likely caused by antagonism of the NMDA receptor.

Although there are many reports of the effect of intrathecal ketamine on different animal models of pain and in clinical trials,[25,26] the specific site and mode of action in the central nervous system is still controversial.[22] The

site of action of the inhibition of the ΔMAP and EMG responses to UBD observed when the drug is administered systemically must be caused by an effect on either the central or peripheral nervous system. The marked and more prolonged inhibition of both responses to UBD observed when the drug is delivered by lumbar intrathecal catheters support a spinally-related analgesic effect of ketamine. A more prolonged effect on baseline MAP in this group could explain part of the more prolonged inhibition of responses. The intrathecal injection of ketamine on the acute reflex responses to colorectal distension was similar and also suggested a spinal antinociceptive effect.[10]

Another possible site of action of the effects of ketamine in our model is on peripheral afferents. There is recent evidence that ketamine and other NMDA antagonists might have effects on NMDA receptors present on the peripheral terminals of primary afferent nerves.[27,28] A peripheral effect of NMDA antagonists in models of visceral pain has not been investigated. The urinary bladder is an organ accessible to topical treatment with several drugs with well-recognized peripheral effects[29] and the local effect of opioids administered intravesically in a model of bladder nociception has been demonstrated.[30] We did not observe inhibition of responses to UBD when ketamine was administered topically into the bladder. In spite of this negative result, a peripheral site of action cannot be ruled out in our preparation. The topical application of the drug into the undamaged bladder does not ensure an adequate delivery of the drug to the nociceptors. Study of the topical administration of ketamine in the damaged bladder (*i.e.*, chemically inflamed) may be of interest.

In the current study we observed that repeated UBD served to activate a sensitization process, since an increase in the vigor of responses after repeated UBD was observed (fig. 5). We have characterized this process in previous reports, although its precise mechanism has not been determined. Kolhekar found a similar increased vigor of visceromotor responses to repeated colorectal distensions. This was prevented by pretreatment with the NMDA receptor antagonist D-APV suggesting a role of the NMDA receptors in the development of visceral hyperalgesia.[5] Ketamine (3 mg/kg) administered as a pretreatment before any distension, failed to prevent the sensitization process, since after 10 UBD, ΔMAP and EMG responses achieved a similar intensity as the group treated with normal saline. However, a delay in the sensitization process for EMG responses to UBD was observed. These results suggest that NMDA receptors may not be related to the sensitization process we have characterized. At the very least, there does not appear to be a preventive effect of ketamine in this model. Our observation that the EMG responses to UBD are initially inhibited after pretreatment with ketamine might be caused by a direct effect of the drug rather than a

prevention of the development of central sensitization. These results, along with the effects of systemic and intrathecal administration of ketamine described previously, strongly suggest that the NMDA receptors might have a role in the acute transmission of nociceptive inputs.

In conclusion, ketamine inhibits, in a dose-dependent manner, cardiovascular and visceromotor responses to the acute noxious stimulus of distension of the urinary bladder. This effect is likely related to NMDA receptor antagonist actions of ketamine, since other drugs with such pharmacologic properties produced a qualitatively similar inhibition. Because of the strong inhibition of the ΔMAP and EMG responses to UBD observed with intrathecal ketamine, the main site of action of the drug appears to be localized to the spinal cord. Ketamine did not affect the development of sensitization associated with the repeated presentation of UBD, although a delay was observed in the EMG responses, probably related to direct effects of the drug. Other mechanisms of hyperalgesia and sensitization (*i.e.*, peripheral mechanisms, other neurotransmitters) rather than the activation of the NMDA receptors will need to be investigated to explain the sensitization process observed in this model of visceral pain. Neurophysiologic studies of spinal neurons responsive to UBD are underway and may serve to delineate more precise mechanisms of drug action.

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