

Cutaneous Amitriptyline in Human Volunteers

Differential Effects on the Components of Sensory Information

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Background: Amitriptyline is effective in relieving neuropathic pain. Its site of action is thought to be supraspinal and spinal, but a peripheral effect on fibers is also suggested.

Methods: This double-blind study examined the effects of transcutaneous amitriptyline diluted in hydroalcoholic solution in healthy young male volunteers. Six treatments were randomly applied on different areas of the skin of the back: amitriptyline at 0 (vehicle), 25, 50, and 100 mm; saline (control); and lidocaine-prilocaine cream as a positive control. Up to 24 h after application, mechanical thresholds for touch and nociception, and thermal thresholds for cold, warm, and heat sensation were recorded for each area. Blood samples were collected to assess plasma levels of amitriptyline. A late recording of the tactile thresholds was performed 1 and 3 weeks after the treatment session.

Results: The thresholds for all sensations did not differ between the vehicle and saline. Lidocaine-prilocaine cream displayed a short-lasting anesthetic effect for all sensations, although this was not significant for warm sensation. Amitriptyline, at the three concentrations studied, induced a mild and short-lasting increase of the tactile and mechanical nociceptive thresholds. It significantly decreased cold thresholds (down to 21.8°C, $P = 0.01$ vs. 27.5°C for control) and heat thresholds (down to 40.1°C, $P = 0.004$ vs. 43.4°C for control). These two effects were no longer significant after the fourth hour of observation. Amitriptyline did not change warm thresholds. There was no apparent systemic absorption effect of the drug.

Conclusion: It is hypothesized that amitriptyline has a differential effect on different fiber structures.

TRICYCLIC antidepressants (TCAs) are effective in relieving neuropathic pain, the best evidence being available for amitriptyline.^{1,2} In animal neuropathic models, TCAs reduce thermal hyperalgesia,³ mechanical allodynia,⁴

and ectopic discharges.⁵ Therefore, TCAs are commonly used for neuropathic pain, where opiates are classically considered as ineffective at the common doses.⁶ However, only 30% of neuropathic patients experiencing pain have a reduction to half of the pretreatment pain level, and the fairly high incidence of side effects could explain the poor compliance for this treatment.^{2,7}

The cellular/synaptic action of TCAs is considered to be largely due to inhibition of reuptake of norepinephrine, serotonin,⁸⁻¹⁰ and adenosine.^{11,12} Other mechanisms have been also suggested, such as blockade of *N*-methyl-D-aspartate receptors,^{13,14} calcium,¹⁵ and sodium voltage-dependent channels.¹⁵⁻²⁰ Animal studies have provided diverse information about the site of action for TCAs on neuropathic pain. Supraspinal effects are supported,²¹ but part of the supraspinal analgesia may be linked to the relief of associated depressive symptoms.²² Spinal effects are also suggested because intrathecal amitriptyline selectively reduces thermal hyperalgesia by spinal ligation,³ and the analgesic effects of clomipramine are reduced by intrathecal injection of opiate receptor antagonists.²¹ Recently, action of TCAs on peripheral nerves has been suggested because (1) locally injected amitriptyline has antinociceptive adenosine-dependent effects on inflammatory and neuropathic animal pain models^{3,23,24}; (2) long-lasting local anesthetic effects have been shown after perineural and transcutaneous amitriptyline in rats and humans²⁵⁻²⁹; and (3) systemic amitriptyline reduces specifically ectopic discharges, which are linked to hyperactivity of sodium channels in the fiber, in the same way as lidocaine.⁵

To understand the mechanisms of action of TCAs on sensory fibers, the effects of transcutaneous amitriptyline on the different components of cutaneous sensitivity were studied according to a protocol already validated in human volunteers.²⁹ These data are preliminary to the potential development of topical TCAs in the treatment of neuropathic pain. As secondary endpoints, the systemic absorption of the drug after this type of application was evaluated, as well as the possible residual effects on the skin.

Materials and Methods

The study was conducted in accordance with the Declaration of Helsinki and was approved by the regional Research Ethics Committee (Comité Consultatif pour la Protection des Personnes se prêtant à la Recherche Biomédicale d'Auvergne, Clermont-Ferrand, France). Healthy

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male patients, aged 18–40, were recruited for the study. This population was chosen to reduce interindividual variability, which could be induced by old age or menstrual cycle. The exclusion criteria were weight out of the 80–120% limits of the Lorentz Ideal Body Weight [$\text{height in cm} - 100 - (\text{height in cm} - 150)/4$]; inability to undergo the psychophysical trials; exclusion period from another clinical trial; acute skin disease; excessively hairy back skin; drug, antidepressive, or excessive alcohol consumption; allergy; and contraindications for amitriptyline, lidocaine, and prilocaine. Written information was given to the patient, and a signed consent was collected. All of the patients had a previous training session for psychophysical training by electronic von Frey.

Amitriptyline hydrochloride was prepared in a vehicle composed of water/isopropanol/glycerin (45/45/10) solution (adjusted to pH 8.5 with sodium hydroxide) by the investigational drug service of the university hospital. Four solutions of amitriptyline were prepared: 0 (vehicle), 25, 50, and 100 mM. The vehicle used was based on that used in similar studies involving lidocaine or amitriptyline.^{29,30} The concentrations were chosen in a range thought to have anesthetic effects,²⁹ with the aim of a total dose (see next paragraph) not exceeding the therapeutic range. The prepared solutions were stored at 4°C in a dark, locked refrigerator. The use of the solutions for the study did not exceed 1 week, and stability was tested over this period (concentration of amitriptyline by ultraviolet spectrophotometry method). The two other treatments were 0.9% NaCl (saline) and lidocaine–prilocaine cream (EMLA; Astra-Zeneca, Rueil-Malmaison, France); these were stored in the same conditions.

Patients were randomly assigned to one order, in which one of the six treatments (saline, vehicle, 25 mM amitriptyline [AMI₂₅], 50 mM amitriptyline [AMI₅₀], 100 mM amitriptyline [AMI₁₀₀], and EMLA) was allocated to one skin area (numbered 1–6). Because each area was to receive 1.5 ml of the solution (or 2 ml EMLA, which is the recommended amount for 18 cm²), the total doses of active drugs for one session were 82.4 mg amitriptyline, 50 mg lidocaine, and 50 mg prilocaine. Randomization was done by a research assistant who was not involved in the observations, and the allocation was kept in a sealed envelope.

Every day of study, two patients were admitted to the research unit at 8:00 AM and were examined for noninvasive hemodynamic parameters (blood pressure and heart rate lying at rest and then in the orthostatic position). A venous cannula was inserted in the arm, and a first blood sample was drawn for amitriptyline assay. A plastic pattern perforated with windows displaying the future areas of treatment was set medially on the back of the patient lying in the prone position, its superior end at the level of the spine of scapula. The borders of the six application areas (numbered 1–6 from left to right and top to bottom) were drawn with a dermatographic pencil

through the windows. After application of cutaneous alcoholic antiseptic, each area (6 × 3 cm) was bordered by a stuck framework made of precut hydrocolloidal sticking plaster (Algoplaque; URGO, Chenôve, France) to avoid the diffusion of the products outside the area. At T0, the six treatments were applied. In one of the areas, determined by randomization, 1.5 ml EMLA was applied by syringe. On each of the other five marked areas, a 6 × 3-cm piece of sterile gauze was placed, and the gauze was saturated *via* syringe with 1.5 ml saline, vehicle, and amitriptyline solutions. Overlapping occlusive dressings (Tegaderm, 9 × 10 cm; 3M Santé, Cergy-Pontoise, France) were fixed to the skin. After 1 h, the dressings and gauze were removed (T1), and each area was lightly wiped with absorbent tissue. Because skin redness has been reported with amitriptyline,²⁹ redness was scored (0–10) after visual comparison to a handmade toner (with Microsoft Office PowerPoint 2003; Redmond, WA). To mask the possible redness and to ensure double-blind testing, each area was then painted with eosin diluted in normal saline, and the border of the area was drawn again with dermatographic pencil. The opening of the envelope for randomization, the application of the treatments, the assessment of redness, the removal of dressings, and the eosin application were performed by the same investigator, who did not participate in the rest of the study.

The subjects were allowed to move freely in the clinical investigation center between observations and had a standard lunch at midday. At the times T1, T1 + 2 h, T1 + 4 h, T1 + 6 h, T1 + 8 h, and T1 + 24 h, blood samples for amitriptyline dosage were drawn, hemodynamic data and general tolerance were noted, and psychophysical studies were performed. Sensory testing was performed in the following order: touch, mechanical nociception, cold, warm, and heat. For each test, the areas were examined in the same order (1–6). Each kind of monitoring was performed by a dedicated examiner. For the mechanical tests, the subject sat on a stool, and the examiner sat behind. For thermal testing, the subject lay on a bed in a prone position. After the T1 + 8 h examination and checking of discharge criteria, the venous cannula was withdrawn and the subject was allowed to go home. Subjects returned to the research unit at T1 + 24 h. The last blood sample was drawn by direct venous puncture.

The tactile threshold for mechanical static (punctate) stimuli was assessed using calibrated (0.008–300 g/mm²) von Frey filaments (Bioseb, Chaville, France). Care was taken to avoid stroking the skin with the hair and to apply only a pressure stimulus. The sensations evoked by touching hair were not taken into account. Filaments were applied to the designated point on the skin for approximately 1 s. von Frey hair applications were separated by at least 5 s to reduce the likelihood of anticipatory responses. The von Frey filaments were

applied in ascending order of stiffness. Tactile threshold was defined as the smallest force (g/mm^2) necessary to bend a von Frey hair, which was just perceived as touch for three consecutive times. If tactile pain threshold exceeded hair number 6.65 (300 g/mm^2), the sensitivity was censored at that number. Because the scale of the threshold values naturally displays an exponential pattern, a natural logarithmic transformation was made on the measured values for further analysis.

The mechanical pain threshold was assessed using an electronic algometer (electronic von Frey; Bioseb). The strain gauge was connected to a plastic sterile cone (Eppendorf, Hamburg, Germany), the tip of which was applied perpendicularly to the studied skin area. The punctate pressure was gradually increased with a constant slope under visual control of the pressure value up to the detection of mechanical pain threshold. Threshold was defined as the lowest pressure that produced a sensation of pain. Each threshold value was averaged from three separate consecutive measurements at different points in the test area. A cutoff value was set at 500 g/mm^2 , which was the considered threshold value if no pain appeared at this cutoff.

The Samedic Thermostest apparatus (Somedic AS, Stockholm, Sweden) was used to deliver quantified and reproducible heat impulses *via* a thermode to the skin area. The thermode, which was initially set in the middle of the area, was maintained with a loose elastic band surrounding the chest. The thermode temperature could be varied between 10° and 52°C and cools or warms depending on the direction of current applied. Before each test, the resting temperature was set to 30°C (temperature of the neutral skin). The temperature of the thermode was then decreased or increased until the volunteer felt the predetermined sensation (cold, warm, or painful heat). At this point, the decrease or increase in temperature was terminated by the volunteer pressing the handheld button. The temperature attained was recorded by the apparatus, and the thermode temperature returned rapidly to 30°C . This procedure was repeated on four further occasions, the threshold being the mean of the last three responses calculated by the apparatus. The entire process was then repeated using the next area to be explored. For assessment of the cold, warm, and painful heat sensation thresholds, the rate of decrease or increase in temperature was set at 0.4, 0.2, and 1°C/s , respectively.

The blood samples were analyzed by the Laboratory of Toxicology of the Faculty of Medicine of Clermont-Ferrand. Amitriptyline and nortriptyline plasma levels were determined using a specific high-performance liquid chromatographic method. The limit of quantitation was 2 ng/ml for each analyte.

To search for possible residual effects of amitriptyline, the patients came back to the research unit $8 (\pm 1)$ and $21 (\pm 2)$ days after the main session for skin examination.

The same pattern was used for the identification of the areas. Only the tactile threshold for mechanical static stimuli was assessed using calibrated von Frey filaments.

Sample size calculation was undertaken on the expectation of a local anesthetic effect of the active drugs. This was quantified as an increase in the mechanical pain threshold ($+80 \text{ g/mm}^2$) on the electronic von Frey. The mean \pm SD for a similar population examined in our research unit is $107 \pm 5 \text{ g/mm}^2$. With initial $\alpha = 5\%$ and $\beta = 10\%$, the sample size was calculated to be 14, considering that each subject was his own control. It was increased to 16 to allow for randomization after a crossover design.

Statistical Analysis

All of the data collected were numeric and were expressed as median and interquartile range. However, the values for redness (scoring on a scale from 0 to 10) and tactile threshold (\log_e of the measured value in grams) were considered as ordinal data. The normality of the distribution for the other data were checked by the Shapiro-Wilks test. For each parameter, the effects of period, subject, area, and treatment were analyzed by an analysis of variance (or a Friedman test for ordinal data). If a significant difference appeared between treatments ($P < 0.05$), a *post hoc* analysis was made by a Tukey test (or multiple pair comparison for ordinal data). The statistical analysis was performed with Statistical Analysis Software 9.1 (SAS Institute Inc., Cary, NC), Microsoft Office Excel 2003, and XLStat (Addinsoft, Paris, France). Figures were generated using Microsoft Office Excel 2003, Microsoft Office Paint 2003, and Microsoft Office PowerPoint 2003.

Results

Of the 16 subjects who underwent the study, one of them was excluded for analysis (except for safety parameters) because he showed major difficulties in concentration during the main session. This was confirmed by further analysis of the thermal threshold data, which showed significant intraindividual discrepancies. For the remaining 15 male subjects, the demographic data were as follows (mean \pm SD): age, $25.4 \pm 4.5 \text{ yr}$; weight, $73.0 \pm 5.4 \text{ kg}$; height, $178.6 \pm 6.5 \text{ cm}$. The baseline hemodynamic data at rest were as follows (mean \pm SD): heart rate, $67.2 \pm 13.4 \text{ beats/min}$; systolic arterial pressure, $133.7 \pm 10.2 \text{ mmHg}$; diastolic arterial pressure, $64.0 \pm 8.5 \text{ mmHg}$. After rapid orthostatism, there was no significant change in the hemodynamic parameters, except for the diastolic arterial pressure, which increased to $75.1 \pm 10.0 \text{ mmHg}$ ($P < 0.00001$).

Throughout the study, the hemodynamic parameters remained stable ($P > 0.05$, analysis of variance for repeated measures). One subject, just before the observa-

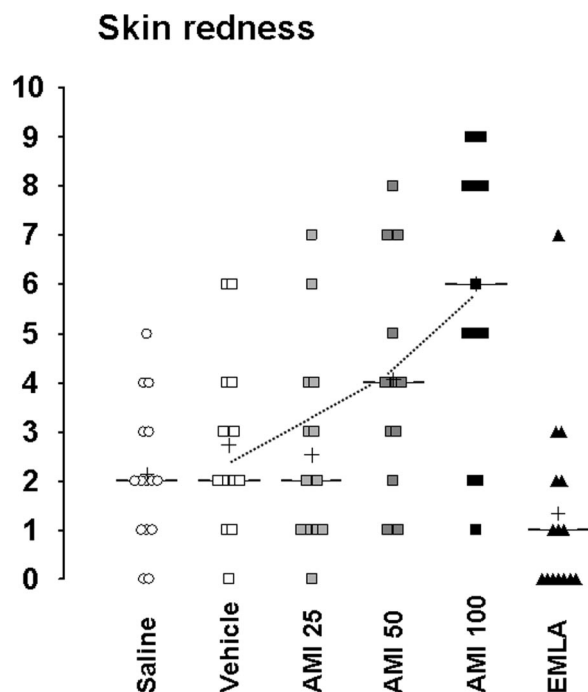


Fig. 1. Scattergrams showing the level of skin redness compared with a dedicated scale, at the end of the application period of the treatments. A significant difference was found ($P < 0.0001$, Friedman test) between treatments. White circles = saline; white squares = vehicle; medium gray squares = 25 mM amitriptyline (AMI); dark gray squares = 50 mM amitriptyline; black squares = 100 mM amitriptyline; black triangles = EMLA (Astra-Zeneca, Rueil-Malmaison, France). A dose effect is suggested (dotted line = trend based on logistic regression), although not statistically significant.

tion time T1 + 6 h, had malaise with sudation, weakness, paleness, and mild hypotension (99/59 mmHg) with a normal heart rate. Hypoglycemia was diagnosed (2.37 mm), and the malaise disappeared 5 min after oral sugar administration. One other subject, 5 min after sitting on the stool at the T1 + 6 h observation, had malaise with paleness, hypotension (80/40 mmHg), and bradycardia (38 min⁻¹). Glycemia was normal (4.84 mm), and vasovagal malaise was diagnosed. This disappeared after 5 min in a lying-down position. In both cases, the electrocardiogram and the amitriptyline blood level were checked in emergency and showed no abnormality, the levels of amitriptyline being below therapeutic levels. As minor events, dry mouth sensation was noted in 8 of the 96 observations (0.8%) of the main session.

The results for skin color are shown in figure 1. Before they were painted with eosin, the skin areas treated with amitriptyline displayed significant redness ($P < 0.0001$, Friedman test). The comparison of the confidence intervals for the redness scores confirmed that redness was higher with AMI₅₀ and AMI₁₀₀ (compared with saline, vehicle, and AMI₂₅), whereas it was lower with EMLA.

Part of the results for tactile sensation is shown in figure 2. A time effect was noted ($P < 0.0001$), with a linear tendency of lowering the thresholds from T1 (median = 1 g/mm²) to T1 + 24 h (median = 0.4 g/mm²),

in accord with habituation. This time effect was independent of the treatment and was noted with the same tendencies with the inactive treatment saline ($P < 0.0001$). At T1, there was a significant effect of treatment ($P < 0.0001$), the *post hoc* analysis showing higher thresholds with EMLA compared with all of the other treatments. At T1 + 2 h, the effect of treatment was still significant ($P < 0.016$), the *post hoc* analysis showing higher thresholds with AMI₅₀, AMI₁₀₀, and EMLA compared with the other treatments. At T1 + 4 h, the significance level was still reached ($P = 0.017$), but the *post hoc* analysis did not allow us to individualize groups. No effect of the treatment was noted at the later observation times.

Part of the results for mechanical nociceptive sensation is shown on figure 2. A time effect was noted ($P < 0.0001$), with a linear tendency of decreasing the thresholds from T1 (mean = 357 g/mm²) to T1 + 8 h (mean = 217 g/mm²) and a mild increase at T1 + 24 h (mean = 239 g/mm²), in accord with habituation. This time effect was partly dependent on treatment, because the same tendency was observed with the saline treatment, without reaching significance. At T1, there was a significant effect of the treatment ($P < 0.0001$), the *post hoc* analysis showing higher thresholds with EMLA compared with all of the other treatments. At T1 + 2 h, the effect of treatment was still significant ($P < 0.002$), the *post hoc* analysis showing higher thresholds with AMI₅₀, AMI₁₀₀, and EMLA compared with the other treatments. No effect of the treatment was noted at later observation times.

Part of the results for cold sensation is shown in figure 3 and 4. No time effect (independent of treatment) was noted. At T1, there was a significant effect of the treatment ($P = 0.01$), the *post hoc* analysis showing lower thresholds (*i.e.*, lower sensitivity) with AMI₂₅, AMI₅₀, AMI₁₀₀, and EMLA (mean values = 23.5°, 21.8°, 22.6°, and 20.3°C, respectively) compared with saline and vehicle (mean values = 27.5° and 27.1°C, respectively). The significance level was not reached at later observation times because the threshold increased to normal values in most subjects. However, the thresholds with amitriptyline remained very low at T1 + 2 h and T1 + 4 h in some subjects (fig. 4), and this effect was weaker with EMLA (fig. 3).

Part of the results for warm sensation is shown on figure 3. No time effect was noted ($P = 0.215$). At T1, the analysis of variance did not show any significant treatment effect ($P = 0.113$), but there was a tendency for higher thresholds with EMLA (mean = 36.8°C) compared with all of the other treatments (mean value for saline = 34.8°C). No effect of the treatment was noted at the later observation times.

Part of the results for heat sensation is shown in figures 3 and 4. No independent time effect was noted. At T1, there was a significant effect of the treatment ($P =$

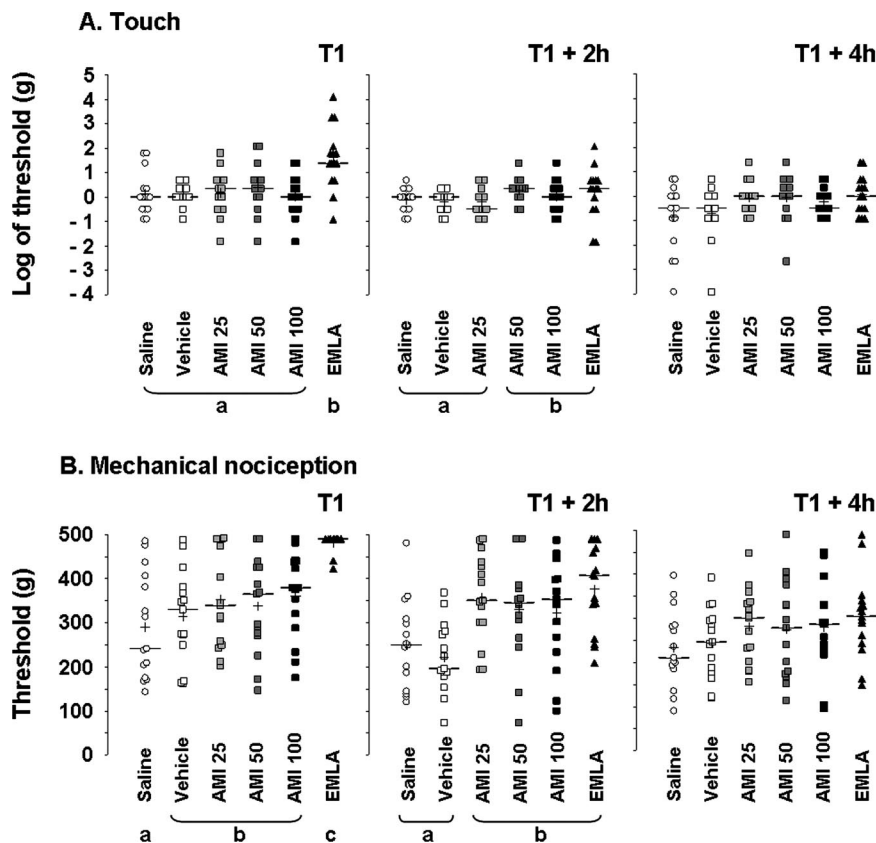


Fig. 2. Scattergrams showing the tactile (von Frey hairs; A) and nociceptive (electronic von Frey; B) cutaneous thresholds after application (T1) of the treatments, in time. Horizontal line = median value; cross = mean value. The data observed after T1 + 4 h do not appear, because no significant difference between the groups (Friedman test for touch and analysis of variance for mechanical nociception) was noted after this time. White circles = saline; white squares = vehicle; medium gray squares = 25 mM amitriptyline (AMI); dark gray squares = 50 mM amitriptyline; black squares = 100 mM amitriptyline; black triangles = EMLA (Astra-Zeneca, Rueil-Malmaison, France). The letters a, b, and c signal the groups identified by the *post hoc* analysis (multiple pair comparison or Tukey test).

0.004), the *post hoc* analysis showing lower thresholds (*i.e.*, higher sensitivity, hyperalgesia) with AMI₂₅ (mean = 40.7°C), AMI₅₀ (40.1°C), and AMI₁₀₀ (40.1°C) compared with EMLA (42.8°C), saline (43.4°C), and vehicle (43.8°C). The same effect was observed at T1 + 2 h ($P = 0.012$). The significance level was not reached at later observation times, as the threshold decreased to normal values in most subjects, but there was still a tendency to difference with amitriptyline at T1 + 4 h (fig. 3). However, the thresholds with amitriptyline remained low at T1 + 2 h and T1 + 4 h in some subjects (fig. 4).

At T1 + 24 h, T1 + 1 week, and T1 + 3 weeks, the tolerance remained excellent. No significant redness or other cutaneous abnormalities were noted at the treated areas, except a mild eczematous reaction to the Algo-plaque (only at T1 + 24 h). The tactile thresholds were identical in all areas ($P = 0.27, 0.39$, and 0.064 at T1 + 24 h, T1 + 1, week and T1 + 3 weeks, respectively).

At all times and for every subject, blood sample analysis did not show plasma levels of amitriptyline and nortriptyline over the detection threshold of 2 ng/ml. This indicated that no relevant systemic absorption of amitriptyline occurred during the study.

Discussion

It is believed that part of the analgesic action of TCAs on neuropathic pain is the result of a peripheral effect. If

this is the case, it may be hypothesized that TCA drugs may be effective when administered in the peripheral field of the involved nerve. This is suggested by animal studies in which locally injected amitriptyline had antinociceptive effects on neuropathic pain models (dorsal root ligation).^{3,24} However, until now, the attempts of relieving patients' neuropathic pain with cutaneous antidepressants have been inconclusive because amitriptyline was not superior to the placebo,^{31,32} whereas some analgesic effects were reported with doxepin.³³

The long-lasting local anesthetic properties of TCAs have been described in preclinical studies^{25–28} and confirmed in human volunteers.²⁹ In this last study, amitriptyline hydrochloride was prepared as a 45/45/10 water/isopropanol/glycerin solution (adjusted to pH 8.5 with sodium hydroxide). The vehicle used was based on one used in a similar study aimed to test lidocaine.³⁰ Each subject (randomized and blinded) received 0.3 ml of four solutions—0, 10, 50, and 100 mM amitriptyline in vehicle—on the ventral aspect of the upper arm with an occlusive dressing. As the mean pain rate decreased from 8 out of 10 to 4–5 out of 10 at the puncture of the treated area, the authors concluded that amitriptyline induced local anesthesia (*i.e.*, suppression of sensations). At the effective concentrations (greater than 50 mM), redness of the skin was often observed. The development of amitriptyline as a local anesthetic could be compromised by reports of axonal damage after perineu-

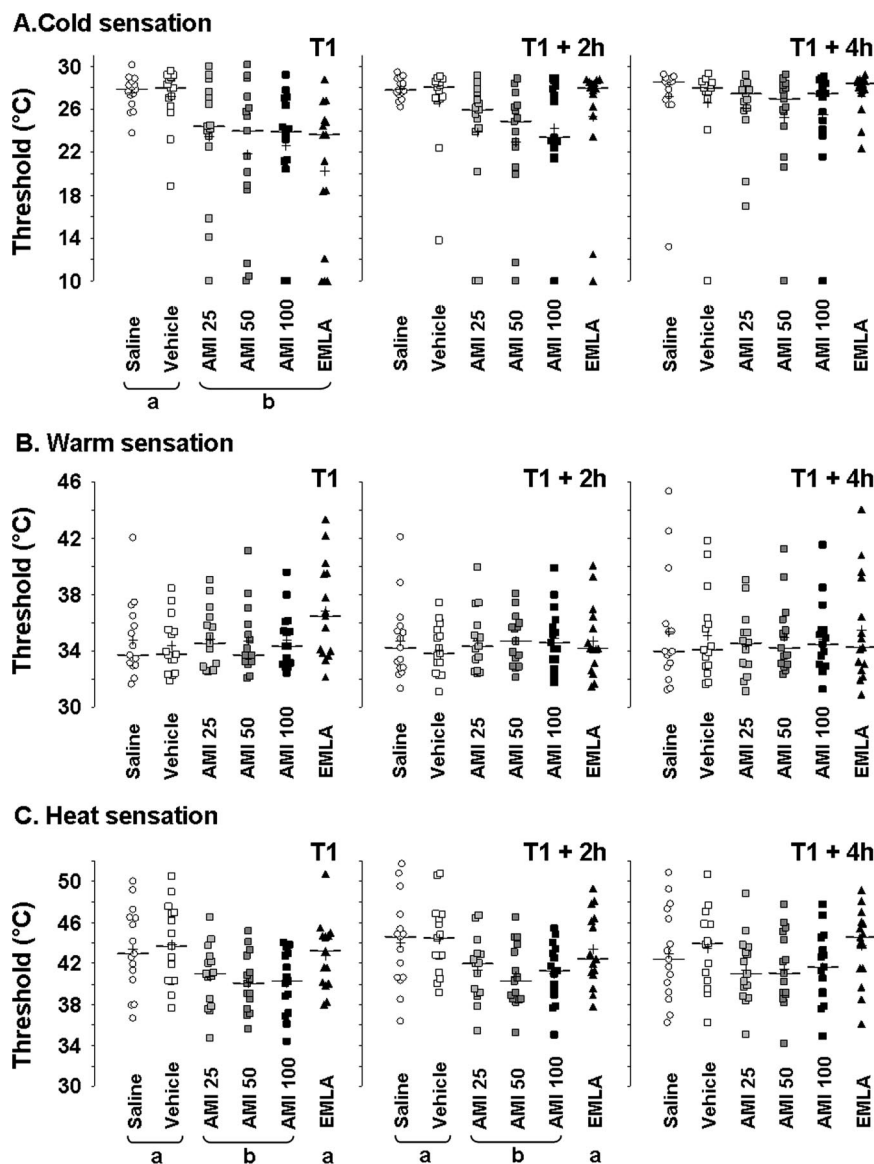


Fig. 3. Scattergrams showing the three kinds of cutaneous thermal thresholds (Thermotest; Somedic AS, Stockholm, Sweden) after the end of application (T1) of the treatments, in time (A: cold; B: warm; C: heat). Horizontal line = median value; cross = mean value. The data observed after T1 + 4 h do not appear, because no significant difference between the groups (analysis of variance) was noted after this time. White circles = saline; white squares = vehicle; medium gray squares = 25 mm amitriptyline (AMI); dark gray squares = 50 mm amitriptyline; black squares = 100 mm amitriptyline; black triangles = EMLA (Astra-Zeneca, Rueil-Malmaison, France). The letters *a* and *b* signal the groups identified by the *post hoc* analysis (Tukey test).

ral administration,^{34,35} but the project of transdermal administration remains active because no signs of toxicity have been noted in the study on human volunteers.²⁹ For this reason, it was decided to investigate further, with a first-step study of the mechanisms underlying the observed local anesthesia. A psychophysical approach with measurement of thresholds was chosen because it allows a dissociated analysis of the different components of skin sensitivity and seems to be less subjective than assessment of pain on simple stimulus. Because we were expecting a local anesthetic effect, we chose to use EMLA as a positive control.

The results of the current study were somewhat unexpected because the local anesthetic effects of amitriptyline were fairly weak. This was especially true on mechanical tests, in which the effects of EMLA were obviously stronger, and a significant difference between amitriptyline and control was only noted at T1 + 2 h (fig. 2). Furthermore, a strong dissociative effect on the dif-

ferent thermal thresholds was noted, with no effect on warm, impairment of cold, and enhancement of heat sensitivity (fig. 3). The effects observed after amitriptyline application lasted longer than for EMLA, but they remained shorter than those reported by Gerner *et al.*,²⁹ who noted residual anesthesia at the fifth hour. The discrepancies between the two studies might be explained by different assessment methods or by different study populations (the current study considered only young males). There was also a strong interindividual variability of the sensitivity to amitriptyline regarding cold sensation thresholds. This might be due to differing capacities of the skin to absorb molecules, although amitriptyline *per se* also has unpredictable analgesic effects when administered systemically in neuropathic patients.^{2,7}

The local anesthetic effect of amitriptyline is supported by the ability of the compound to block voltage-dependent sodium channels.¹⁵⁻²⁰ However, such properties cannot explain the dissociation observed in the

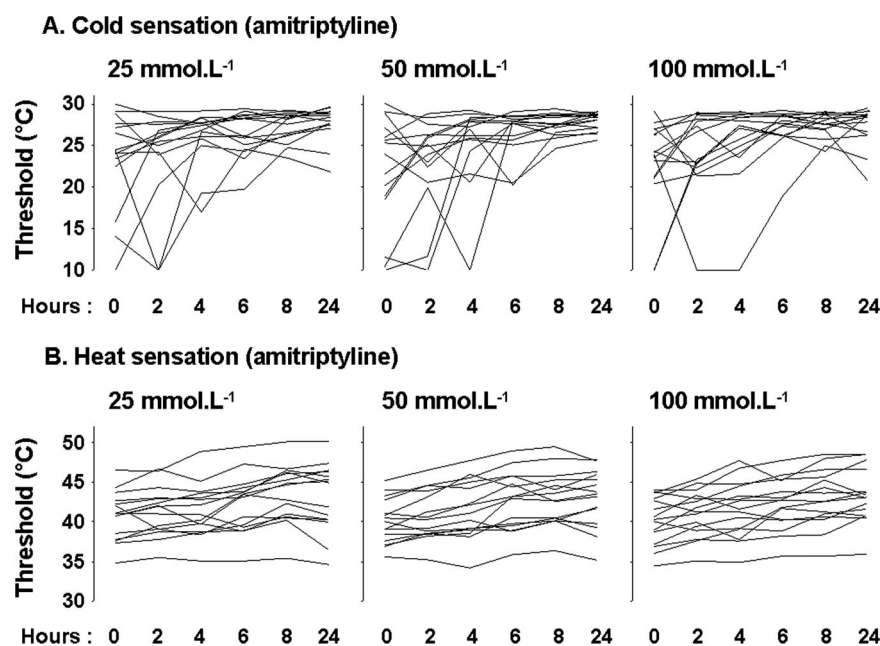


Fig. 4. Time course of the two kinds of cutaneous thermal thresholds (Thermotest; Somedic AS, Stockholm, Sweden) affected by amitriptyline application (A: cold; B: heat). Each line represents one subject. These diagrams show a tendency to return to normal values at T1 + 8 h, as well as a strong interindividual variability of the sensitivity to amitriptyline, regarding cold sensation thresholds.

current study. It must be noted that amitriptyline, like other TCAs, has many possible sites of action on the neurone, such as (1) blockade of histaminergic, adrenergic, serotonergic, muscarinic,³⁶ nicotinic,¹⁵ and glutamatergic^{13,14} receptors; (2) norepinephrine,^{10,37} serotonin,^{10,37} and adenosine^{11,23,24} uptake inhibition; and (3) blockade of voltage-dependent calcium^{15,38,39} and potassium⁴⁰⁻⁴² channels. Some of these properties may explain our results. Furthermore, the interactions between amitriptyline and thermal receptors (TRPV₁₋₃ and TREK for heat, TRPM₈ and TRPA₁ for cold) have not been explored yet.⁴³⁻⁴⁶ The dissociation between the effects on warm and heat sensations can also be explained by the type of fibers, more than by specific receptors. Indeed, it is known from animal studies that (1) temperatures below 29°C activate dedicated A δ fibers ("A-cold"), (2) temperatures between 35° and 42°C (nonnoxious range) activate principally low-threshold A δ fibers and dedicated C fibers ("C-warm"), and (3) temperatures over 41°C (noxious heat) activate multimodal ("C-mechano-heat") fibers.^{43,44,47,48}

The current study is only the first step to investigate a concept and cannot be extrapolated at the moment to neuropathic pain patients. The ability of topical amitriptyline to provide pain relief in these patients still needs to be explored, although inconclusive results have been reported with this molecule diluted in cream.³¹ The different explanations for this failure may be of a physiologic nature, because the site of action may not be the field of the nerve, but the lesioned area. There may also be methodologic reasons, because the patients included in the previously cited study had heterogeneous histories of painful disease, and some of the patients included had already been treated with TCAs orally, without success. Central sensitization may have been involved in the

cases of long-lasting neuropathic pain, explaining the poor effect of a peripheral treatment. Other types of peripheral neuropathic pain (such as touch or cold evoked) can be very disabling, causing distress and suffering for individuals, and these are rarely considered in therapeutic trials.⁴⁹ These could be preferential indications for testing the analgesic properties of topical amitriptyline. Finally, there may be technical reasons for the failure of the study, such as an insufficient concentration (<20 mg/ml) or a bad penetration of the drug through the human skin. Because the skin barrier is made of epidermal cells all linked together by tight junctions, the transdermal penetration is optimal only for lipophilic, nonionized, and unbound molecules. Amitriptyline (molecular weight of hydrochloride = 313.9; pKa = 9.42, nonionized in alkaline medium; logP = 4.64-5.04) has all of these properties, but its penetration is also conditioned by the quality of the vehicle (which must be fat or alcoholic).

Because the transdermal formulation of amitriptyline that we used in the current study seems not to be applicable in the clinical context, creating an acceptable formulation is a necessary step for a future development. The concentration of amitriptyline must be sufficient for good bioavailability at the site of action (*i.e.*, the nerve fiber) without risk of massive systemic absorption. All of these challenges must be raised before starting new clinical trials involving neuropathic patients experiencing pain.

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