Pharmacology Biochemistry and Behavior, Vol. 47, pp. 21-26, 1994
Printed in the U.S.A.

0091-3057/94 $6.00 + .00

Site- and Test-Dependent Antinociceptive Efficacy of Amitriptyline in Rats

R. DIRKSEN,*[1] D. VAN DIEJEN,* E. L. J. M. VAN LUIJTELAAR† AND L. H. D. J. BOOIJ*

**Institute for Anesthesiology and †Department of Psychology, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands*

Received 19 February 1993

DIRKSEN, R., D. VAN DIEJEN, E. L. J.M. VAN LUIJTELAAR AND L. H. D. J. BOOIJ. *Site- and test-dependent antinociceptive efficacy of amitriptyline in rats.* PHARMACOL BIOCHEM BEHAV 47(1) 21-26, 1994.—The antinociceptive efficacy of systemic- (IV), spinal- (IT), and global supraspinal (ICV)-administered amitriptyline (AMIT) was compared in three different tests for nociception: the hot-plate test, the tail-flick test, and the withdrawal reflex test. Systemic AMIT inhibited the responses in each of the three tests, with distinct dose-effect relationships. Spinal AMIT reduced in a dose-dependent fashion the force of withdrawal to noxious electrical stimulation but was ineffective in the hot-plate test and facilitated the responses in the tail-flick test. Supraspinal AMIT inhibited in a dose-dependent fashion the response to the stimulus of the hot plate, reduced the force of withdrawal after a dose that was effective by the IV route, and again facilitated the responses in the tail-flick test. The results suggest that spinal sites mediate the inhibition of the withdrawal reflex and the supraspinal site the inhibition of the hot-plate test. Two conclusion are drawn: First, AMIT's site of action varies among the pain modalities; and, second, augmentation of the reactions can occur. The complex interaction accords with the clinical experience that the benefits of AMIT in pain treatment are hard to predict.

Amitriptyline Intrathecal Intravenous Intracerebroventricular Antinociception Pain Rat

THERAPEUTIC effects of tricyclic antidepressant drugs in the treatment of pain have been reported in many studies (3,15,19,22,29). Among the antidepressant drugs, amitriptyline (AMIT) is often used. Clinical reports show that the analgesic efficacy of AMIT varies extremely (18,20,23,32), and the reason for this is unknown. Also, the mechanisms by which antidepressants may affect chronic pain remain unclear, although Ward et al. (30) suggested that imbalances in biogenic amines in the CNS may modify the modulation of pain in the paleospinothalamic tract. Within this framework, many animal studies are performed. IT injection of AMIT failed to cause antinociception in thermal algesic tests (2,17,26), in contrast to what was anticipated (30). Systemic or ICV administration of AMIT resulted in behaviourally defined antinociceptive effects (1,4,24,25,27,28), while in other studies systemic AMIT was shown to have no effects in acute and chronic experiments (2,13).

This diversity of effectiveness of AMIT in both human and animal studies might be due to the complexity of the pain system (6), with the various emotional and motivational dimensions that exist in the various types of pains. Therefore, it is necessary to use different types of tests of nociception to capture various pain modalities and dimensions. In this study, a comparison is made between global supraspinal (ICV), systemic (IV), and spinal (IT) administration of AMIT in three different tests for nociception. Within the heat modality, two different tests are used because it has been shown that the tail-flick and hot-plate tests will yield different effects following administration of pentazocine (9). A second modality is noxious transcutaneous electrical stimulation that causes a withdrawal reflex (8). For each of these tests and each of these routes of administration, a dose-effect relationship is uncovered.

METHOD

Experimental Animals

Drug-naive male Wistar rats of the outbred strain Cpb WU (CPB-TNO, Zeist, The Netherlands) with a body weight of 250-300 g were used. Preexperimental conditions were standardized. Five animals were housed together in a macrolon cage and received standard food and tapwater ad lib. They were kept on a 12 L : 12 D cycle with white lights on at 8:00 a.m. and the environmental temperature was constantly 21 °C. After chronic cannulation of the subarachnoid or ventricular space, animals were housed separately.

[1] To whom requests for reprints should be addressed.

Preparations

IT preparation. Rats (n = 106) anaesthetised with isoflurane were equipped with a polyethylene catheter (PE-10) into the lumbar subarachnoid space through an opening made into the atlantooccipital membrane according to a procedure described by Yaksh and Rudy (31). Animals showing gross motor disturbances after full recovery from the anaesthesia were discarded. Animals were allowed to recover for 12 days prior to the start of the experiments.

ICV preparation. Rats (n = 69) anaesthetised with isoflurane were placed in a stereotaxic frame for left-sided implantation of a polyethylene cannula (PT-97). The cannula was placed 4.0 mm below the skull surface. According to the coordinate system of König and Klippel (16), the following coordinates were chosen: AP +6.1, L 1.4. After placement, the cannula was fixed to the skull with acrylic dental cement (Paladur). Animals were allowed to recover for 7–10 days.

Drug Injections

The drug administered was amitriptyline HCl (Sigma Chemical Co., The Netherlands). The solvent was NaCl 0.9%. Solutes were freshly prepared shortly before administration. Dosages refer to the salt. Control experiments included the injections of the solvent by routes of administration similar to that of the experimental drug. IT and ICV injections were carried out using a calibrated gear-driven microinjector.

IV injections were performed directly in a tail vein (n = 42) or through the intrajugular catheter (n = 45). The dosages used were: 0.25, 1, 2, 4, 6, 8, and 16 mg/kg. The drug was delivered in a volume of 0.1 ml/kg. The catheter wash was 0.25 ml.

For an IT injection, AMIT was solved in NaCl 0.9%. The dosage used were: 10, 25, 50, and 100 μg. The dose was administered in 10 μl, and the catheter was subsequently flushed with a volume of 10 μl NaCl 0.9% (catheter volume is 7 μl). Six hours after IT injection of AMIT, the position of the cannula was verified by assessing the effectiveness of a single IT dose of 10 μl lidocaine 2%. The data of rats not exhibiting a clear suppression of withdrawal or paralysis of the hind legs after IT injection of lidocaine were excluded.

For ICV injections, AMIT was solved in NaCl 0.9%. The dosages used were: 10, 50, 100, 200, and 300 μg. The dose was administered in 5 μl. The catheter was subsequently flushed with a volume of 10 μl NaCl 0.9% (catheter volume is 7 μl). At the end of an experiment, the position of the cannula was verified by injection of 10 μl of a solution of methylene blue. Results from animals not exhibiting a clear distribution of methylene blue in the ventricular system were excluded.

Tests for Nociception

The three tests of nociception were performed at an environmental temperature of 21°C.

Thermal algesic tests. The thermal algesic tests used were the hot plate and the tail-flick tests and these were performed in this order. The hot plate was maintained at 52.5°C. In this test, the interval was measured between the time the rat was placed on the hot plate and the time it started to lick a hind paw. The tail-flick was evoked by placing the tail of an animal over a slit under which a halogen projection lamp (300 W) was placed. In this test, the interval was measured between the time the light was switched on and the time the tail was flicked away. In both tests, these intervals are known as the response latencies (RLs). If a response was absent, tissue damage was limited by an empirically assessed standard cutoff time of 60 s in the hotplate test and 30 s in the tail-flick test. Response latencies were assessed prior to drug injection and at intervals of 5, 10, 15, 30, 60, 90, 120, and 240 min after injection.

Noxious induced withdrawal reflexes (NIWRs). A rat was anaesthetised with an IP injection of urethane (1.2 g/kg) to allow cannulation of the right internal jugular vein, trachea, and right carotid artery. Anaesthesia was supplemented and maintained by adding IV aliquots of urethane after cannulation of the jugular vein (three doses in 0.5 h and to a total of 0.2 g/kg). The rat was then placed on the experimentation table. The right hind paw was mounted in a shoe that contained two electrodes for bipolar stimulation (Grass Stimulator S88, with stimulus isolation unit SIU 5, and constant-current unit CCU 1A). Stimulation parameters were set to 4-ms pulse duration, 7.5-mA stimulus strength, 100-Hz pulse frequency, in a train of 500-ms duration, and a repetition rate of 12.5 mHz (0.75 min^{-1}) for the trains. The hind paw was connected to a force transducer (Grass FT 03C), which measured the force of the withdrawal response to the electrical stimulus. Determination of drug effects on the withdrawal reflex started 1 h after termination of the surgical preparation. During this interval, parameters were stabilised (8).

Measures

Hotplate and tail-flick tests. The effect was defined as the maximum change within 30 min after injecting the drug. The maximum percentages of effect (MPEs) after injection of the drug were compared with those after injection of saline.

The MPE for each animal was calculated as

$$\text{MPE} = \frac{\text{postinjection RL} - \text{preinjection RL}}{\text{cutoff time} - \text{preinjection RL}} \times 100,$$

where the postinjection response latency is the maximum change in latency after injection of drug or saline. If the postinjection response latency was shorter than the preinjection response latency, the denominator was the preinjection response latency.

NIWRs. The individual baseline withdrawal force (expressed in g) was measured over a 20-min period. The average of the baseline responses was calculated and served to determine the individual relative responses expressed as percentage of this mean baseline response (8). The effect is defined as the difference between the mean of the baseline responses (100%) and the minimum response after injection of the drug.

Statistical Analysis

The percentages of effect were analysed with a one-way analysis of variance (ANOVA). If appropriate, a test was supplemented with Scheffé's method for multiple comparisons. A difference yielding a p value of less than 0.05 was considered significant. Each data point is derived from measurements in a minimum of six rats and is given as mean and SEM. The equation of the sigmoid E_{max} model (14) was fitted to the dose–response data to obtain three parameter estimates (E_{max} = maximum effect; γ = exponent that determines the shape of the curve; and ED_{50} = dose causing 50% of the effect).

RESULTS

Predrug Responses and Control Data

The predrug response latencies in the hot-plate test were different for rats that had been implanted with IT or ICV

catheters (21 ± 1 or 23 ± 1 s, respectively) and those without (17 ± 1) (ANOVA, $p < 0.01$). The predrug tail-flick response latencies were not different among rats with or without an indwelling catheter. The coefficients of variation were of a similar magnitude for the hotplate and tail-flick test (38 and 27%, respectively) and substantially lower for the NIWR test that uses the anaesthetised rats (6%). Because the baseline response latencies in the hot-plate test were different, it was decided to compare the relative change in response to injection of AMIT (% effect). Injection of solvent by the IV, IT, or ICV route did not result in responses different from preinjection values.

IV Administration of AMIT

After IV injections of AMIT, the response latencies in the hot-plate and tail-flick tests were increased (ANOVA, $p < 0.0001$). The magnitude of the withdrawal response to noxious electrical stimulation was likewise inhibited (ANOVA, $p < 0.0001$). The dose-effect relationship is provided by the parameter estimates obtained by fitting the data of each test and each route of administration to the equation of the sigmoid E_{max} model (14).

Figure 1 shows the relationships between the dose and the inhibitory effect of IV AMIT. The three curves are obtained by fitting the data to the equation of the dose–effect relationship. The parameter estimates γ (the exponent) and ED_{50} for each test are shown in Table 1. The E_{max} reached the maximum of 100% in all tests and is therefore omitted in Table 1. The ED_{50} was different for each test of nociception. The γ of the dose-effect relationships in the hot-plate and tail-flick tests was different from the NIWR ($p = 0.03$ and 0.011, respectively).

AMIT caused also a reduction in the blood pressures of anaesthetised rats ($p < 0.0001$). Fitting the equation to the data on the diastolic blood pressures yielded the parameter estimates E_{max} (40 ± 17% reduction), ED_{50} (0.7 ± 0.6 mg/kg), and the exponent (1.1 ± 1.4). The parameter estimates of fitting the equation to the data of the systolic blood pressures were: E_{max} of 35 ± 9% reduction; ED_{50} 1.0 ± 0.6 mg/kg; and the exponent 1.2 ± 0.7. Other miscellaneous effects of IV injections included: a short period of sitting motionless after injection of 8 mg/kg and almost instantaneous respiratory arrest, cyanosis, and death in the unanaesthetised rat after injection of 16 mg/kg. The latter dose caused cardiovascular collapse and death in the artificially ventilated and anaesthetised rat.

TABLE 1

PARAMETER ESTIMATES (±SE OF THE ESTIMATE) OF FITTING THE EQUATION OF THE SIGMOID E_{max} MODEL TO THE DATA OF IV AMITRIPTYLINE

Parameter estimate	HP	TF	NIWR
γ	2.9 ± 0.6*	3.7 ± 0.8*	1.6 ± 0.1
ED_{50}	1.9 ± 0.1°	6.0 ± 0.3°	1.0 ± 0.04°

HP, hotplate test; TF, tail-flick test; NIWR, noxious induced withdrawal reflex; γ, parameter that determines the shape of the dose-response curve (*different from that in the NIWR); ED_{50} (mg/kg; different from the other tests).

IT AMIT

AMIT in a dose of 10, 20, 25, or 50 μg did not result in a change of the hot plate response latencies in the first 30 min after IT injection ($p = 0.2$) (Fig. 2). The tail-flick response latencies were reduced after injection of these dosages ($p = 0.004$). Posthoc evaluation showed a difference between vehicle- and drug-treated animals but no difference among the doses. The responses varied to a greater extent than predrug, as evidenced by a coefficient of variation that ranged between 40 and 80%.

Slight changes in motor control after IT injection of 50 μg were indicated by perturbed motor coordination; however, during handling muscle strength appeared to be normal. IT injection of 75 and 100 μg resulted in the inability of animals to support the hind part of the body and this lasted 20 and 30

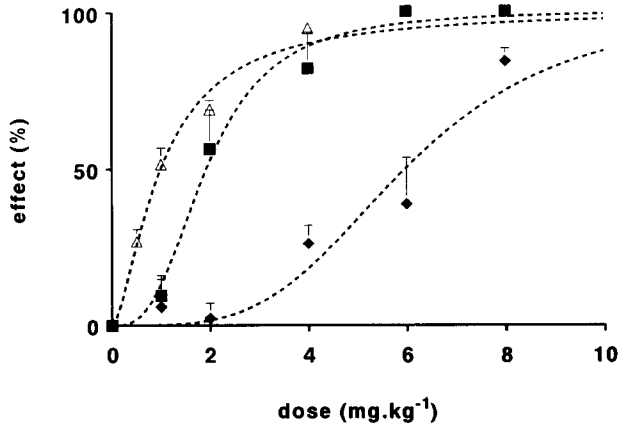

FIG. 1. Dose-response relationships of IV amitriptyline in three tests for nociception. It shows inhibition of the responses: elicited by electrical stimulation (-△-); in the hotplate test (-■-); and in the tail-flick test (-◆-). Each symbol represents the mean of data obtained from measurements in a minimum of six rats; the bars represent the SEM (parameter estimates of the dose-response curves are given in Table 1).

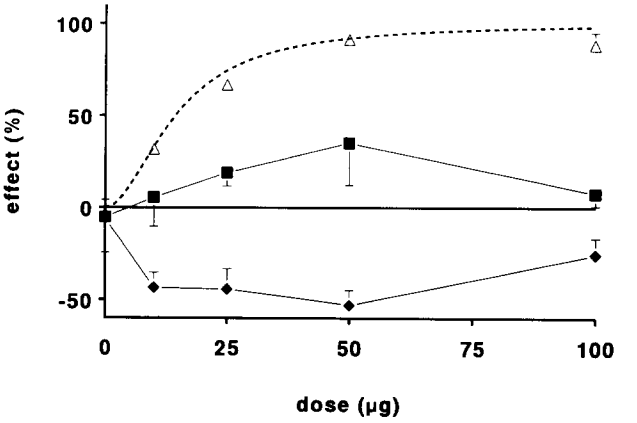

FIG. 2. Dose-response relationships of IT amitriptyline in three tests for nociception. The equation of the sigmoid E_{max}-model is fitted to the data on the inhibition of the response elicited by electrical stimulation (-△-). The data on the responses in the hotplate test (-■-) and in the tail-flick test (-◆-) are presented in line graphs. Each symbol represents the mean of data obtained from measurements in a minimum of six rats; the bars represent the SEM.

min, respectively ($p < 0.01$). The response latencies at recovery from impaired motor control after injection of 100 μg were not different from the baseline latencies.

Approximately 60–90 min after IT injections of AMIT and irrespective of the dose, the hot plate response latencies had increased to 60 s (cutoff time). At this time, the tail-flick response latencies were not different from baseline values.

The responses to noxious electrical stimulation were inhibited in a dose-dependent fashion ($p < 0.0001$) (Fig. 2). The estimate of γ (1.9 ± 0.4) is of the same magnitude as that for IV AMIT. The ED_{50} is 14.4 ± 1.9 μg, which is 20-fold lower than the ED_{50} after IV AMIT.

The magnitude of the reduction of the blood pressures depended on the dose ($p < 0.003$). The diastolic blood pressures had reduced with 5 ± 10, 28 ± 5, 30 ± 7, and 39 ± 3% after 10, 25, 50, and 100 μg, respectively. These dosages caused a reduction of the systolic blood pressures of 5 ± 4, 12.5 ± 2, 13 ± 3, and 18 ± 2%, respectively.

ICV AMIT

ICV injections of AMIT resulted in a dose-dependent increase of the hotplate response latencies ($p < 0.001$) (Fig. 3). The ED_{50} (100 ± 30 μg) is fivefold lower than that of IV AMIT. The parameter estimate of the exponent (1.4 ± 0.5) is of the same magnitude as that derived from using the NIWR test.

The tail-flick response latencies were reduced in a dose-dependent fashion (behaviourally defined "hyperalgesia") ($p < 0.001$) (Fig. 3). The change of hot plate response latencies started within 5 min and that of the tail-flick test after an interval of 15 min.

ICV injections resulted in a decrease of the magnitude of the force of withdrawal elicited by noxious electrical at the dose of 300 μg ($p = 0.0035$) but not after injections of 10 or 100 μg (Fig. 3). The magnitude of effect after ICV or IV administrations of 300 μg is not different (42 ± 8, and 52 ± 5%, respectively; $p = 0.31$). The magnitude of the reduction

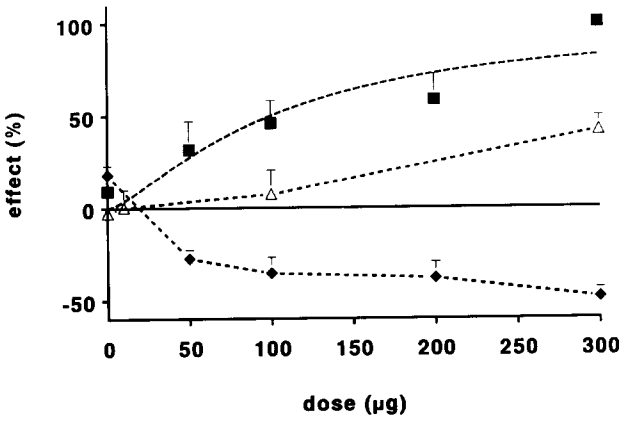

FIG. 3. Dose-response relationship of ICV amitriptyline in three tests for nociception. The equation of the sigmoid E_{max}-model is fitted to the data on the inhibition of the responses to the stimulus of the hotplate (-■-). The data on the responses elicited by electrical stimulation (-△-) and the responses elicited in the tail-flick test (-◆-) are presented in line graphs. Each symbol represents the mean of data obtained from measurements in a minimum of six rats; the bars represent the SEM.

TABLE 2

SUMMARY OF THE OUTCOME OF THE THREE TESTS FOR NOCICEPTION IN RELATIONSHIP TO THE ROUTE OF ADMINISTRATION OF AMIT

	Heat		Electrical
	Tail-Flick Test	Hotplate Test	NIWR Test
Systemic (IV)	↓	↓	↓
Supraspinal (ICV)	↑	↓	↓*
Spinal (IT)	↑	–	↓

↑augmented response (behaviourally defined hyperalgesia); ↓inhibited response (behaviourally defined analgesia), (*) only after a dose that has the same effect by the systemic route; –no change of the responses.

of the blood pressures depended on the dose ($p < 0.003$). The diastolic blood pressures had reduced with 20.1 ± 9.6, 19.2 ± 7.6, and 46.3 ± 3.4% after 10, 100, or 300 μg, respectively. These dosages caused a reduction of the systolic blood pressures of 5 ± 3, 14 ± 3, and 20 ± 4%, respectively.

Table 2 gives a summary of the effects in the three tests of nociception in relationship to the route of administration.

DISCUSSION

AMIT is generally considered to be an agent that can be effective in the treatment of pain (29). The general outcome of the present study accords with the presence of intrinsic analgesic effects after systemic administration of AMIT. The reactions to different types of noxious stimuli were found to be consistently inhibited by systemic AMIT in a dose-dependent fashion. The inhibition of the responses after AMIT in the hotplate test was earlier found by Tura and Tura (20), and this study adds the relationship between dose and inhibitory effect. The inhibition of the responses elicited in the tail-flick test was dependent on the dose of AMIT and this confirms results of earlier studies (5,24,25,28). AMIT inhibits in a dose-dependent fashion the responses in the NIWR test, which suggests that the inhibitory effect is not limited to the heat modality of pain. The results in the NIWR test are similar to those induced by other types of analgesics such as nicomorphine, morphine, and pentazocine (9,11,12).

That systemic AMIT has a consistent inhibitory effect in each of the three tests of nociception is not counterpart of sameness of dose-effect relationship. The mathematical analysis yielded different values of the parameter estimates γ and ED_{50}, which shows that each test of nociception results in a distinct dose-effect relationship. In addition, IT and ICV injections of AMIT proved to have differential effects in each of the three tests of nociception. Thus, we found that AMIT has distinct dose-effect relationships *and* different sites of action when different modalities of pain are used. The sites of action and effects of IT and ICV AMIT are discussed further.

It was found that AMIT may take effect at a spinal or supraspinal site. Whether the first or the latter site is involved seems to depend on the type of test. In the hot-plate test, ICV AMIT inhibits the responses; in contrast, IT AMIT is not effective. When the NIWR is used to test nociception, it is found that IT injections results in a dose-dependent inhibitory effect. In this test, only the ICV injection of the highest dose (300 μg) of AMIT has an effect. However, the body weight of

rats is 250–300 g and accordingly the 300-μg dose is close to 1 mg/kg. Because the systemic injection of 1 mg/kg AMIT results in a similar effectiveness and lower dose by ICV route proved ineffective, distant rather than local effects are involved. Together, we propose that the NIWR is inhibited by spinal sites of action.

The tail-flick test yields results at variance. Earlier studies have reported on the absence of an inhibitory effect in this test after systemic or IT AMIT (2,26), which in itself is not contradictory with the antinociceptive potency of a substance (9). However, our data show that both IT and ICV injection of AMIT result in shortened and scattered response latencies, suggesting behaviourally defined hyperalgesia. The augmentation of the responses in the tail-flick test after IT and ICV AMIT is opposite to the inhibition after the systemic injections, and the presence of counteracting effects might be related to the finding that the ED_{50} of AMIT in the tail-flick test is substantially higher (and thus the efficacy lower) compared to that in the two other tests for nociception.

Two effects observed during the experiments remain to be discussed: first, the delayed effect after IT AMIT in the hotplate test; and, second, the reduction in the blood pressures. The first effect is a dose-independent increase of the hotplate response latencies that occurred approximately 60–90 min after IT injection of AMIT. At this interval, the responses of the tail-flick or NIWR tests were not different from baseline values. The independency of dose and this effect may indicate a nonspecific phenomenon. Another possibility is that AMIT, or metabolites, migrates from the spinal site of injection to supraspinal sites. The rostrad ascend of intrathecally injected drugs was earlier proposed to explain delayed supraspinal effects in rats after IT injection of somatostatin (10) or bupivacaine (7). Noteworthy, the supraspinal actions of ICV AMIT have likewise caused a change of the response latencies in the hot-plate test, but not an inhibitory effect in the two other tests.

AMIT is known to cause cardiovascular side effects (21). In our study, AMIT causes a relevant and dose-dependent reduction of blood pressures after IT, ICV, or IV drug injections. Both IT and ICV injections cause hypotensive effects, which indicates that this effect of AMIT is caused by an action not confined to the spinal or supraspinal site. This contrasts with the site dependency of the antinociceptive effects. Moreover, the responses in the tail-flick test were enhanced after the same IT or ICV doses of AMIT that caused relevant reductions of blood pressures. This means that the actions of AMIT that result in changes of blood pressures and nociception are separate. Yet, the cardiovascular effects of AMIT explain a difference in results in our and two earlier studies. The earlier studies reported dwindling down of effect at increasing IP dose (24,28), whereas such a phenomenon was absent in our study. Cardiovascular perturbation is known to affect drug uptake from the site of injection into the blood. The direct injection into the blood used in our study avoids the impact of the uptake kinetics of a drug.

In conclusion, systemically administered AMIT results in a dose-dependent antinociceptive effect in each of the three tests of nociception. The effectiveness of amitriptyline in the hotplate test relies on an inhibitory effect at the supraspinal level, whereas the inhibition of a withdrawal reflex elicited with transcutaneous electrical stimulation relies on a spinal inhibitory effect. IT or ICV injections result in a facilitated response in the tail-flick test, which means that the mechanism of an inhibitory effect after IV injections is a different one.

ACKNOWLEDGEMENTS

The help of W. Jansen and F. van der Pol (zoological technicians) and W. Kleinhans (electrotechnical technician) of the Institute for Anesthesiology of the University of Nijmegen is gratefully acknowledged. This study was performed in the Animal Research Laboratory of the Institute for Anesthesiology.

REFERENCES

1. Ardid, D.; Guilbaud, G. Antinociceptive effects of acute and "chronic" injections of tricyclic antidepressant drugs in a new model of mononeuropathy in rats. Pain 49:279–287; 1992.
2. Botney, M.; Fields, H. L. Amitriptyline potentiates morphine analgesia by a direct action on the central nervous system. Ann. Neurol. 13:160–164; 1983.
3. Butler, S. Present status of tricyclic antidepressants in chronic pain therapy. In: Benetti C.; et al., eds. Advances in pain research and therapy. vol. 7. New York: Raven Press; 1984:173–198.
4. Butler, S. H.; Weil-Fugazza, J.; Godefroy, F.; Besson, J.-M. Reduction of arthritis and pain behaviour following chronic administration of amitriptyline or imipramine in rats with adjuvant-induced arthritis. Pain 23:159–175; 1985.
5. DeFelipe, M. C.; DeCeballos, M. L.; Fuentaes, J. A. Hypoalgesia induced by antidepressants in mice: A case for opioids and serotonin. Eur. J. Pharmacol. 125:193–199; 1986.
6. Dirksen, R. Analgesiology: A new approach to pain. In: Parris, W. C. V., ed. Contemporary issues in chronic pain management. Boston, MA: Kluwer Academic Publishers; 1991:33–51.
7. Dirksen, R.; Lerou, J.; Bakker, H.; Luijtelaar, E. v.; Nijhuis, G.; Gielen, M.; Booij, L. The dose–response and time–effect relationships of intrathecal bupivacaine in rats. The influence of epinephrine and pH. Acta Anaesthesiol. Scand. 36:153–158; 1992.
8. Dirksen, R.; Lerou, J.; Lagerwerf, A.; Vree, T.; Nijhuis, G. M. M.; Booij, L. H. D. J.; Jurna, I. A small animal model for pharmacological studies of general anesthetic agents. Eur. J. Anaesth. 7:285–298; 1990.
9. Dirksen, R.; Lerou, J.; Nijhuis, G. M. M.; Booij, L. H. D. J. Pentazocine: Not an agonist/antagonist analgesic. Eur. J. Anaesth. S3:1–50; 1990.
10. Dirksen, R.; Lerou, J.; Nijhuis, G. M. M.; Booij, L. H. D. J.; Jurna, I. Intrathecal somatostatin produces effects depending on the interval between catheter implantation and drug injection. Life Sci. 47:1347–1354; 1990.
11. Dirksen, R.; Pinckaers, J. W. M.; Egmond, J. v.; Tielbeek, E. A.; Nijhuis, G. M. M. Indications for perispinal opiates. In: Gomez, Q. J.; Egay, L. M.; Cruz-Odi, M. F., eds. Anaesthesia: Safety for all. Amsterdam: Elsevier Science Publishers B.V.; 1984:397–407.
12. Dirksen, R.; Pol, F. v. d.; Nijhuis, G. M. M. Mechanisms of effectiveness of intravenous nicomorphine and its hydrolysis products in rats. Br. Med. J. special issue:10–17; 1988.
13. Godefroy, F.; Butler, S. H.; Weil-Fugazza, J.; Besson, J.-M. Do acute or chronic tricyclic antidepressants modify morphine antinociception in arthritic rats? Pain 25:233–244; 1986.
14. Holford, N. G.; Sheiner, L. B. Kinetics of pharmacological response. Pharmacol. Ther. 16:143–166; 1982.
15. Kessler, K. A. tricyclic antidepressants: Mode of action and clinical use. In: Lipton, M. A.; Dimascio, A.; Killam, K. F. Psychopharmacology: A generation of progress. New York: Raven Press; 1978:1289–1303.
16. König, J. F. R.; Klippel, R. A. The rat brain: A stereotaxic atlas of the forebrain and lower parts of the brain stem. Baltimore, MD: Williams & Wilkins; 1963.
17. Larsen, J.-J.; Arnt, A. Spinal 5-HT or NA uptake inhibition potentiates supraspinal morphine antinociception in rats. Acta Pharmacol. Toxicol. 54:72–75; 1984.

18. Levine, J. D.; Gordon, N. C.; Smith, R.; McBryde, R. Desipramine enhances opiate postoperative analgesia. Pain 27:45–49; 1986.
19. Magni, G.; Conlon, P.; Arsie, D. Tricyclic antidepressants in the treatment of cancer pain: A review. Pharmacopsychiatry 20:160–164; 1987.
20. Pilowsky, I.; Hallett, E. C.; Bassett, D. L.; Thomas, P. G.; Penhall, R. K. A controlled study of amitriptyline in the treatment of chronic pain. Pain 14:169–179; 1982.
21. Raisfeld, I. H. Cardiovacsular complications of antidepressant therapy. Am. Heart J. 83:129–133; 1972.
22. Romano, J. M.; Turner, J. A. Chronic pain and depression: Does the evidence support a relationship? Psychol. Bull. 97:18–34; 1985.
23. Rosenblatt, R. M.; Reich, J.; Dehring, D. Tricyclic antidepressants in treatment of depression and chronic pain. Anesth. Analg. 63:1025–1032; 1984.
24. Sacerdote, P.; Brini, A.; Mantegazza, P.; Panerai, A. E. A role for serotonin and beta-endorphin in the analgesia induced by some tricyclic antidepressant drugs. Pharmacol. Biochem. Behav. 26:153–158; 1987.
25. Spiegel, K.; Kalb, R.; Pasternak, G. W. Analgesic activity of tricyclic antidepressants. Ann. Neurol. 13:462–465; 1983.
26. Taiwo, Y. O.; Fabian, A.; Pazoles, C. J.; Fields, H. L. Potentiation of morphine antinociception by monoamine reuptake inhibitors in the rat spinal cord. Pain 21:329–337; 1985.
27. Tura, B.; Tura, M. S. The analgesic effect of tricyclic antidepressants. Brain Res. 518:19–22; 1990.
28. Ventafridda, V.; Bianchi, B.; Ripamonti, C.; Sacerdote, P.; DeConne, F.; Zecco, E.; Panerai, A. E. Studies on the effects of antidepressant drugs on the antinociceptive action of morphine and on plasma morphine in rat and man. Pain 43:155–162; 1990.
29. Walsh, T. D. Antidepressants in chronic pain. Rev. Clin. Neuropharmacol. 6:271–295; 1983.
30. Ward, N. G.; Bloom, V. L.; Dworkin, S.; Fawcett, J.; Narasimhachari, N.; Friedel, R. O. Psychobiological markers in coexisting pain and depression: Towards a unified theory. J. Clin. Psychiatry 43:32–39; 1982.
31. Yaksh, T. L.; Rudy, T. A. Chronic catheterisation of the spinal subarachnoid space. Brief communications. Physiol. Behav. 17: 1031–1036; 1976.
32. Zitman, F. G.; Linssen, A. C. G.; Edelbroek, P. M.; Stijnen, T. Low dose amitriptyline in chronic pain:the gain is modest. Pain 42:35–42; 1990.