

INFLUENCE OF DOXEPIN USED IN PREEMPTIVE ANALGESIA ON THE NOCICEPTION IN THE PERIOPERATIVE PERIOD. EXPERIMENTAL AND CLINICAL STUDY

*Jerzy Wordliczek, Marcin Banach, Magdalena Dorazil,
Barbara Przewłocka*,#*

Department of Anaesthesiology and Intensive Care, 1st Chair of General Surgery of Collegium Medicum,
Jagiellonian University, Kopernika 17, PL 31-501 Kraków, Poland, *Department of Molecular Neuropharmacology,
Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Influence of doxepin used in preemptive analgesia on the nociception in the perioperative period. Experimental and clinical study. J. WORDLICZEK, M. BANACH, M. DORAZIL, B. PRZEWŁOCKA. Pol. J. Pharmacol., 2001, 53, 253–261.

The aim of the present research was to assess in experimental and clinical study the influence of doxepin administered intraperitoneally (*ip*) as preemptive analgesia on the nociception in the perioperative period. The pain thresholds for mechanical stimuli were measured in rats. The objective of clinical investigation was to assess the influence of preemptive administration of doxepin on postoperative pain intensity, analgesic requirement in the early postoperative period as well as an assessment of the quality of postoperative analgesia by the patient.

Doxepin injected *ip* (3–30 mg/kg) dose-dependently increased the pain threshold for mechanical stimuli measured in paw pressure test in rats. Doxepin injected 30 min before formalin significantly increased the nociceptive threshold in the paw pressure test. In contrast, doxepin injected 240 min before formalin or 10 min after formalin did not change the nociceptive threshold. Morphine administered subcutaneously (*sc*) at a dose of 1 mg/kg increased the pain threshold measured in the paw pressure test 55 min after formalin treatment. Injection of 10 mg/kg of doxepin 30 min before formalin further enhanced the response after morphine administration. The results of the clinical study demonstrated that the patients who were administered doxepin preemptively showed significantly lower pethidine requirement in order to achieve a similar level of postoperative analgesia.

The results of the research under discussion confirm the theoretical assumptions that there is a possibility to modify the nociception process in the perioperative period through preemptive analgesia using a drug that modifies the activity of the descending antinociceptive system.

Key words: *doxepin, nociceptive threshold, formalin test, rat, pethidine requirement, VAS, PCA system, patients*

correspondence

Abbreviations: CGRP – calcitonin gene related peptide, EAA – excitatory amino acids, NMDA – N-methyl-D-aspartate, PCA – patient-controlled analgesia, PP test – paw pressure test, SP – substance P, TCA – tricyclic antidepressant, VAS – visual-analogue pain assessment scale, WDR – wide dynamic range cells

INTRODUCTION

Pain stimulation related to operative trauma results in a modified response of the nervous system, which causes hypersensitivity to pain stimuli both in the operative lesion (primary hyperalgesia) and in surrounding lesion-free tissues (secondary hyperalgesia). In order to avoid such changes, a strategy has been developed to prevent hyperalgesia from developing in the postoperative period [29, 33]. Such a mechanism is known as preemptive analgesia and involves the modification of the nociceptive process starting in the pre-operative period. In the perioperative period this process consists of two phases [32]. Phase one is directly related to nociceptive stimulation accompanying tissue injuries inflicted during the operation, whereas phase two appearing after the operation, is the result of inflammatory response of injured tissues and changes in the nociceptive structures of the dorsal horn caused by phase one.

Special possibilities of preemptive analgesia for modifying the nociceptive process are offered by modulating the activation of the descending antinociceptive system, especially the adrenergic and serotonergic pathways.

Noradrenergic neurons are found in diencephalon, pons and medulla projecting into the cerebral cortex, into the I, II, IV–V layer of the dorsal horn as well as into the front horn of the spinal cord. Adrenergic α_2 receptors are located in the spinal dorsal horns. Presumably, their activation is essential in order to affect opioid analgesia [11]. Moreover, the activation of the presynaptic receptors by noradrenaline inhibits the release of pronociceptive neurotransmitters, while the stimulation of postsynaptic receptors directly inhibits the activity of neurons in dorsal horns [7, 19].

Serotonergic neurons are present mainly in the nucleus raphe and project both into the periaqueductal grey substance (PAG), hippocampus and into the cerebral cortex, as well as I, II and V

layer of the dorsal horns [6]. Serotonergic neurons, projecting into the dorsal horns, inhibit postsynaptically the neurons from the spino-thalamic tract [nociceptive and wide dynamic range cells (WDR)], probably inhibit the presynaptic release of substance P (SP) and calcitonin gene related peptide (CGRP) from the central primary afferents and through the 5-HT₃, activate GABA interneurons in dorsal horns of the spinal cord [5, 8].

Among antinociceptive drugs that activate the descending antinociceptive system are, among others, tricyclic antidepressants (TCA) [18]. They act through both raising the level of noradrenaline and/or 5-HT in noradrenergic and serotonergic structures of the descending antinociceptive system (through inhibiting secondary uptake of those neurotransmitters), and potentiating opioid analgesia [15, 20, 25, 28]. TCA are also important as modulators of some effects mediated by NMDA receptors. It is well known that NMDA receptors activation is one of the primary factors conditioning the development of the nociception process [30]. In the presented experiments doxepin, which belongs to the class of TCA was used in order to induce the preemptive analgesia. Doxepin also demonstrates strong anxiolytic action, which makes it especially useful in the perioperative period.

The aim of the experimental research was to assess the influence of doxepin administered intraperitoneally (*ip*) on pain thresholds for mechanical stimuli in rats (assessment of specific antinociceptive action of the drug under investigation). Also we assessed the influence of the drug on morphine activity and on the development of pain-related behavior in the formalin model of inflammatory response following prior administration of doxepin. The objective of clinical investigation was to assess the influence of preemptive administration of doxepin on postoperative pain intensity, analgesic requirement in the early postoperative period, frequency of side effects as well as an assessment of the quality of postoperative analgesia in a patient.

MATERIALS and METHODS

Animal study

Male Wistar rats, weighing 250–320 g, were housed in groups of eight to a cage under a constant light-dark cycle (light on between 08.00 and 20.00 h), with free access to food and water. The experiments

were carried out according to the protocol approved by the Ethical Commission of the Institute of Pharmacology.

Nociceptive threshold

Nociceptive threshold was evaluated using a paw pressure (PP) test (Randall-Selitto test, Ugo Basile). An animal was gently restrained and an incremental pressure was applied *via* a piston onto the dorsal surface of the hind paw. The cut-off pressure was 480 g. The measurements were taken 3 times at 15-second intervals, and their mean was used for calculations. Before the experiment nociceptive threshold (baseline) was evaluated for every rat and doxepin was injected 5 min later at doses of 3, 10 and 30 mg/kg *ip* ($n = 27$; 9 rats for each dose). The PP test was conducted 30 and 55 min after doxepin administration.

Formalin model

In order to mimic clinical surgical procedures in which nociceptive stimulation takes place, in our experiments the formalin model in rats was used [10, 14, 27]. The rats were lightly anesthetized with halothane (halothane, 2–3 vol % oxygen, 5 l/min in the special Plexiglas cage) and 100 μ l of 10% formalin solution was subcutaneously (*sc*) injected into the dorsal surface of the left hind paw, according to method described by Malmberg and Yaksh [21]. General anesthesia was used because of two reasons: ethical – since the injection of formalin induces a very strong nociceptive stimulation, and secondly – in order to mimic the surgery procedures in clinic. Three types of experiments were performed with the formalin model. In the first one, the nociceptive threshold was measured using PP test 30 and 55 min after formalin injection. In the second experiment, the intensity of formalin-induced pain-related behavior was quantified by counting the incidence of spontaneous flinching, shaking and jerking of the injected paw for each individual

animal for 60 min. The number of pain-induced behaviors was scored for two characteristic time points: 0–5 (first phase) and 20–40 min (second phase) after formalin administration. In the third type of experiment, the antinociceptive effect of morphine was measured in the PP test 55 min after formalin injection.

The effect of doxepin on nociceptive threshold after formalin treatment was measured. Before the experiment nociceptive threshold (baseline) was evaluated for every rat and doxepin at a dose of 10 mg/kg *ip* was injected 5 min later; 30 ($n = 10$) or 240 ($n = 6$) min after doxepin administration, the rats were lightly anesthetized with halothane and 100 μ l of 10% formalin solution was injected *sc* into the dorsal surface of the left hind paw. In one group of rats ($n = 6$) doxepin was injected 10 min after formalin. The nociceptive threshold was measured 55 min after formalin administration. The effect of doxepin on formalin-induced pain-related behavior was measured according to criteria described above.

The effect of doxepin injected at a dose of 10 mg/kg *ip* ($n = 6$) 30 min before formalin injection on antinociceptive action of morphine was also measured. Morphine was injected at a dose of 1 mg/kg *sc* 55 min after formalin injection. The measurements of antinociceptive threshold in the PP test were conducted 15 and 30 min after morphine administration. Control animals were injected in the same way with physiological saline and tested with the same time schedule as experimental groups.

The results were statistically assessed by an analysis of variance (ANOVA). Inter-group differences were analyzed by Duncan's multiple-range test.

Clinical study

The clinical study involved 40 cholecystectomy patients of 1st Chair of General Surgery of Collegium Medicum, Jagiellonian University (Tab. 1).

Table 1. Patient characteristics

	Number of patients	Sex M/F	Age (years)	Body mass (kg)	Duration of procedure (min)	Observation time (h)
Control	20	18/2	41.7 \pm 7.7 (30–57)	67.5 \pm 8.9 (59–83)	79.65 \pm 15.28 (60–115)	19.7 \pm 1.9 (17–22)
Doxepin	20	17/3	40.2 \pm 8.1 (25–58)	65.7 \pm 7.4 (61–80)	83.15 \pm 14.56 (65–120)	20.4 \pm 1.5 (16–23)

The patients qualified for the investigation were from 18 to 60 years old, and did not suffer from any diseases of the respiratory system, cardiovascular system, kidney or liver problems. Overweight or emaciated patients were excluded from the study.

An additional qualifying criterion for the participation in the study was the inclusion of psychological tests determining the neurotism level (Eysenck test) and anxiety (Spielberger test). Patients whose neurotism level (N) was between 1 and 7 stens, extroversion level (E) was between 4 and 7 stens, the lying level (K) did not exceed 7 stens, the anxiety level (state) was between 22 and 79% while the anxiety level (feature) was between 21 and 81% were admitted to the study. An additional qualifying criterion was the so-called anticipated postoperative pain intensity (VAS E) [34]. The test was conducted in the patients before the operation. The patients answered the question: "what do you imagine pain intensity to be after the operation?" using the visual-analogue pain assessment scale (VAS). Patients whose anticipated pain intensity was between 5 and 7 qualified for further study.

Subsequently, patients were assigned at random to two study groups. One day before the operation, patients in the doxepin group ($n = 20$), were administered orally 75 mg of doxepin (3×25 mg), while patients in the control group ($n = 20$) were administered placebo. The operation was performed under general anesthesia ($O_2 - 33\% + N_2O - 66\%$), muscle relaxation was maintained by pancuronium in fraction doses, while anesthesia during the operation was intensified with fentanyl (doses of 100 to 200 μ g). After the operation, none of the studied patients was administered opioid antagonists.

After the operation, patients were connected to a PCA system, which enabled them to adjust postoperative analgesia. After PCA activation, patients in both study groups were administered 20 mg of IV pethidine at doses of 2 ml of 0.9% NaCl, with the PCA cut off period timed for 10 min.

In all patients the postoperative pain intensity was measured using VAS [26] three times: VAS_R – postoperative pain intensity which made the patients activate the PCA system for the first time, VAS_1 – postoperative pain intensity after 5 to 6 h following the surgery, VAS_2 – postoperative pain intensity in the morning within the first 24 h after the surgery. The total pethidine requirement in the early postoperative period (within the first 24 h) was also determined in all patients studied. The fre-

quency of undesirable side effects (drowsiness, nausea, vomiting) in the early postoperative period (24 h) was monitored. Moreover, the patients were also asked to assess the quality of postoperative analgesia (very good, good, satisfactory, unsatisfactory).

Results of the study were then subjected to a statistical analysis using Student's *t*-test. The value of $p < 0.05$ demonstrated a statistically significant result. In the event of two groups being compared, analysis of variance was conducted. To evaluate differences between groups, the Newman-Keuls test for multiple comparisons was used. In order to statistically analyze the frequency of side effects and assessment the analgesia method by the patients, the independence test χ^2 was used.

RESULTS

Animal study

Doxepin injected *ip* (3, 10 or 30 mg/kg) dose-dependently increased the pain threshold for mechanical stimuli measured in paw pressure test. The effect of two higher doses (132 and 141%, respectively) was significant 30 min after their injection, but 55 min after doxepin treatment the effect was still present and even more pronounced (161%) when the highest dose was used (Fig. 1).

Doxepin injected 30 min before formalin significantly increased (120%) the nociceptive threshold in the paw pressure test (Fig. 2A). In contrast, doxepin injected 240 min before formalin (Fig. 2B)

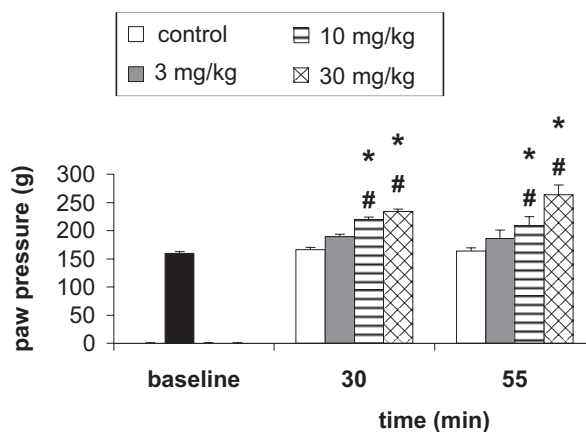


Fig. 1. The effect of doxepin at doses of 3, 10 and 30 mg/kg ($n = 9$ for each dose) administered *ip* on nociceptive threshold measured 30 and 55 min after injection in paw pressure test in rat, * $p < 0.02$ vs control group, # $p < 0.01$ vs baseline measurement

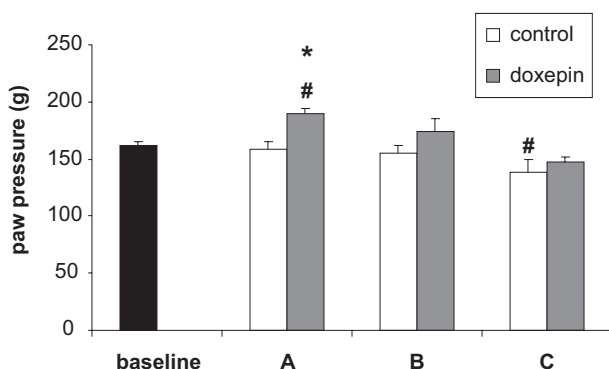


Fig. 2. The effect of 10 mg/kg of doxepin administered *ip* 30 min (A, n = 10) and 240 min (B, n = 6) before, or 10 min after (C, n = 6) injection of formalin on nociceptive threshold investigated in rats in the paw pressure test 55 min following the formalin injection (means \pm SEM), * $p < 0.05$ vs control group, # $p < 0.05$ vs baseline measurement

or 10 min after formalin did not change the nociceptive threshold (Fig. 2C). No effect of doxepin on formalin-induced pain behavior was observed, regardless of the time interval between the injections of doxepin (30, 240 min before or 10 min after formalin) and formalin. The measurements were made during first phase (5–10 min) and three times in the second phase (40–45, 45–50 and 50–55 min). The last two time intervals are not shown (Tab. 2).

Table 2. The effect of 10 mg/kg of doxepin administered *ip* 30 min (A) and 240 min (B) before, or 10 min after (C) injection of formalin on formalin-induced pain behavior, measured during first (5–10 min) and second phase (40–45 min). The results are presented as means \pm SEM of the number of spontaneous flinches, shakes and jerks of the formalin-injected paw from 8–10 animals in group A and 6 animals in groups B and C

Group		5–10 min	40–45 min
A	Control	40.3 \pm 2.7	47.4 \pm 3.1
	Doxepin	44.5 \pm 2.8	46.7 \pm 2.6
B	Control	40.1 \pm 3.5	45.3 \pm 3.0
	Doxepin	43.7 \pm 3.9	46.0 \pm 3.9
C	Control	42.8 \pm 6.5	43.8 \pm 3.3
	Doxepin	40.5 \pm 3.2	45.1 \pm 3.9

Morphine at a dose of 1 mg/kg *sc* increased (130%, 30 min after morphine) the pain threshold measured in PP test 55 min after formalin treatment. Injection of doxepin (10 mg/kg *ip*) 30 min before formalin further enhanced (147%) the response 30 min after morphine administration (Fig. 3).

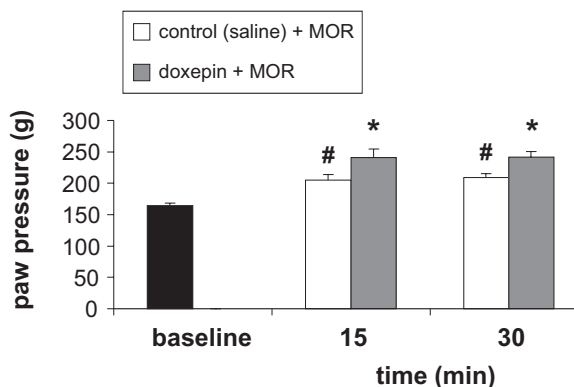


Fig. 3. The effect of doxepin (10 mg/kg, n = 6) administered *ip* 30 min before the injection of 10% formalin solution, on the nociceptive threshold (means \pm SEM) investigated in rats in the paw pressure test 15 and 30 min following morphine (MOR) administration. Morphine (1 mg/kg) was injected *sc* 55 min following the formalin administration, * $p < 0.02$ vs control group, # $p < 0.001$ vs baseline measurement

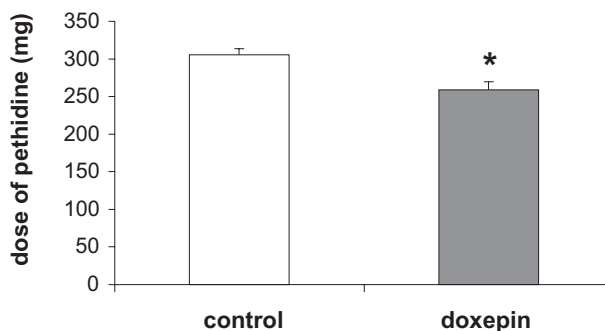


Fig. 4. The effect of preemptive administration of doxepin (75 mg *po*, n = 20) on pethidine requirements in the early postoperative period (mean \pm SEM), * $p < 0.05$ vs control group, (n = 20)

Clinical study

The total pethidine requirement in the early postoperative period was significantly lower (85%) in the patients who had been administered doxepin before the operation as compared with the control group (Fig. 4). Average values of postoperative pain intensity (VAS_E, VAS_R, VAS₁ and VAS₂) were similar in both groups and no statistically significant differences between those groups were observed (Fig. 5).

The results demonstrate that patients who were administered doxepin pre-emptively showed significantly lower pethidine requirement in order to achieve a similar level of postoperative analgesia. No significant differences were observed regarding

the frequency of undesirable side effects or the assessment of the quality of postoperative analgesia by the patients.

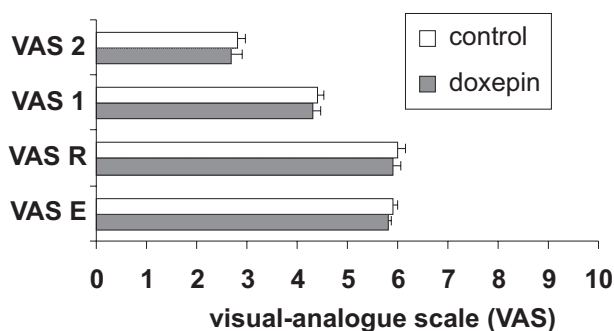


Fig. 5. The effect of preemptive administration of doxepin (75 mg *po*, $n = 20$) on the postoperative pain level in the early postoperative period (mean \pm SEM). In all patients the pain intensity was measured using the visual-analogue pain assessment scale (VAS) four times: VAS_E – anticipated postoperative pain intensity, VAS_R – postoperative pain intensity which made the patients activate the PCA system for the first time, VAS₁ – postoperative pain intensity after 5 to 6 h following the surgery, VAS₂ – postoperative pain intensity in the morning within the first 24 h after the surgery

DISCUSSION

The flow of nociceptive information in the perioperative period has a two-phase character and is in agreement with behavioral changes observed in the formalin-induced inflammatory pain model (postoperative) [1, 4, 13, 27]. Therefore, such a model was used in the experimental part of the investigation. In the formalin pain model nociceptive stimulation is tonic, which closely reflects the postoperative pain character that accompanies tissue injuries. It allows to conduct pain sensitivity tests during tissue injury as well as to study central and peripheral sensitization. Neurochemical changes that accompany the injury, such as the release of pronociceptive transmitters, substance P (SP) and excitatory amino acids (EAA), can be reflected in the test. It was found that *sc* injection of formalin caused nociceptive stimulation whose duration and intensity was sufficient for the appearance of elevated levels of SP and EAA in dorsal horn structures, connected with the process of nociception [16]. It was also demonstrated that following the use of formalin both the expression of mRNA that encodes preprotachykinin and *c-fos* happened at

time intervals related to their respective functions in the processes of nociception [17, 23].

Like in postoperative pain, inflammation induced by locally administered formalin is characterized by two phases of increased pain sensitivity in rats. Phase one lasts from 0 to 10 min and phase two from 10 to ca. 120 min. They are characterized by behavioral symptoms induced by increased nociceptive stimulation. The animals demonstrate decreased mobility, they attempt to protect the paw into which formalin was injected (paw-raising, licking or paw-shaking and bending). These symptoms are correlated with increased neuronal response determined in electrophysiological studies [31]. Phases of the formalin-induced inflammation differ also with respect to their neurochemical aspects. Phase one is connected with direct stimulation of nociceptors due to increased secretion of mainly SP, bradykinin and EAA. Phase two shows an elevated level of histamine, prostaglandins, 5-HT and bradykinin, which leads to the development of a local inflammatory response as well as progressive functional changes in the spinal cord, and then, at higher levels of the nervous system [12, 27]. Hence, pain-induced behavior, which appears in the formalin model, is among other things, an exponent of the development of peripheral and central sensitization processes.

In the present study, the formalin-based postoperative pain was induced in rats following halothane anesthesia. Halothane was used due to the lack of its significant influence on the development of the processes of sensitization [2, 14, 16], although in animals under study the sensitization symptoms (paw raising, shaking or licking) peaked not after 40–45 min following formalin injection, but at later stages: 45–50 and 50–55 min. This was probably due to the influence of halothane and the observation is in agreement with reports by other authors [24].

Doxepin used in the study is a TCA, which acts both through the activation of the descending antinociceptive system and through potentiating opioid analgesia, and also presumably as modulators of some effects mediated by NMDA receptors.

In order to facilitate referring our investigations to clinical studies in which doxepin was administered orally, in experimental studies it was administered *ip* at doses of 3, 10 and 30 mg/kg. The injection of doxepin induced a significant increase in the pain threshold for mechanical stimuli, with the ex-

ception of the dose of 3 mg/kg. For further experiments, the dose of 10 mg/kg was chosen.

In order to determine the optimal time for drug administration, doxepin was administered *ip* 30 or 240 min before formalin injection. It was determined that *ip* doxepin administered preemptively, i.e. 30 min before the formalin injection, significantly raised the pain threshold for mechanical stimuli, which testifies to the possibility of doxepin-based modulation of the activity of the descending antinociceptive system, both in supraspinal centres and at the spinal level. Such an effect was not present in rats receiving doxepin 240 min before the formalin injection in order to reflect the time interval (necessary for the appearance of antinociceptive action of doxepin) used in clinical studies. The absence of doxepin activity is probably related to the fact that the rat eliminates the drug faster, which is due to different rate of metabolism (as compared with man) and is directly related to the long time interval elapsing between the drug administration and the injury. The absence of doxepin activity in rats, that were administered it 10 min after the formalin-based pain was induced, confirms the hypothesis that the modulating influence of doxepin does not appear when sensitization mechanisms are developing or have developed. The studies show that in order to activate the endogenous antinociceptive systems, doxepin should be administered *ip* before the injury occurs, i.e. before the development of phase one of the formalin-induced postoperative pain model.

Doxepin did not influence the pain-induced behavior, which suggested that the development of the sensitization processes was not completely inhibited, but only reduced, since the pain thresholds were increased. This finding remains in agreement with results or research into the antinociceptive properties of other TCA, amitriptyline [3, 28] and desipramine [15, 25]. Goldstein et al. [15] observed, both an increased morphine level in blood serum and an increased level of analgesia in a group of rats treated with desipramine. It may be related to common biotransformation pathways for both drugs in which cytochrome P-450 participates. A higher level of unmetabolized morphine is thus the cause of the opioid binding with more opioid receptors [15]. Ventafridda et al. [28] compared, among others, the influence of chlorimipramine, amitriptyline, nortriptyline and trazodone, administered with morphine, on pain thresholds in rats and

observed that only chlorimipramine and amitriptyline intensified the analgesic effect of morphine. Likewise, Sawynok and Reid [25] proved that desipramine, which itself did not demonstrate internal analgesic activity, intensified opioid analgesia.

Our own studies demonstrated small, but statistically significant (by ca. 15%) reduction of pethidine requirement in patients receiving doxepin. The effect may be related to both the activation of the descending antinociceptive system (inhibition of secondary synaptic uptake of noradrenaline and 5-HT) by doxepin and the inhibition of opioid metabolism by TCA [15]. Moreover, one of the pethidine metabolites (norpethidine) demonstrates its own analgesic activity, which may influence pain intensity in the patients (its lowest level was observed within 24-hour postoperative period VAS2). Equally significant is also the fact that the patients who received doxepin before the operation reported approximately the same postoperative pain intensity (measured on the VAS scale) as compared with the patients in the control group.

The results of our research are in partial agreement with clinical observations of Levine et al. [20], who observed not only the lengthening of the time interval, but also increased analgesic activity of morphine used following the administration of desipramine. Chapman and Butler [9] compared the analgesic properties of doxepin and placebo against pain induced by irritating dental pulp in volunteers. Both were used for the period of 30 days at doses of 150 mg before performing the actual nociceptive test. No analgesic activity of doxepin was observed. Kerrick et al. [18], who used TCA (amitriptyline) for three days after the operation (phase two), observed that TCA had no influence on postoperative morphine requirement and on the overall well-being of the patients. Small reduction in pethidine requirement and the absence of influence on postoperative pain intensity observed in the study under discussion are probably related to the short period of doxepin administration before the operation (24 h).

Although both experimental and clinical studies demonstrated a relatively small influence of *ip* or orally administered doxepin on the inhibition of sensitization processes, the results of research under discussion confirm the theoretical assumptions that there is a possibility to modify the nociception process in the perioperative period through pre-

emptive analgesia using a drug that modifies the activity of the descending antinociceptive system.

Acknowledgment. Supported by grant No. 4P05C 006 16 from the State Committee for Scientific Research, Warszawa, Poland.

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Received: March 26, 2001; in revised form: May 8, 2001.