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Analgesic Effect of Antidepressant Drugs

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KORZENIEWSKA-RYBICKA, I., A. PŁAŹNIK. *Analgesic effect of antidepressant drugs.* PHARMACOL BIOCHEM BEHAV **59**(2) 331–338, 1998.—Imipramine, amitriptyline, citalopram, and maprotiline were examined in different models of a nociceptive reaction after single-dose, and 21-day long administration, in rats. Animals' behavior in the Porsolt and open-field tests was also studied to compare analgesic and antidepressant-like action of drugs and to check the contribution of changes in the rats' gross behavior to animals' reactions to the nociceptive stimuli. The time- and dose-dependent fluctuations in the blood and brain concentrations of imipramine were evaluated in another group of animals. Imipramine, amitriptyline, citalopram, and maprotiline were shown to exert analgesic activity in some tests only. The most unequivocal analgesic effects were observed in the writhing test (2% acetic acid solution IP). The antinociceptive action of antidepressants in this test was probably not due to their local anaesthetic activity, because it was also present after intragastric drugs administration. Alterations in the open-field behavior of rats subjected to the treatment with antidepressant drugs did not correlate with animals' behavior in the writhing test. In the Porsolt test, the antidespair effects of antidepressants were not observed after acute drugs administration at the doses effective in the writhing test. Moreover, in contrary to the writhing reaction, the antiimmobility effect was potently enhanced after repeated administration of tricyclic drugs. Additionally, no association was found between the blood and brain concentrations of chronically administered imipramine and its effects in the writhing test. The obtained results indicate: (a) disparate sensitivity to antidepressant treatment of differently evoked behavioral reactions to the nociceptive stimuli; (b) the most potent effects of administered antidepressants in the model of visceral pain; (c) a better correlation of the brain concentration of imipramine with its antiimmobility than analgesic effect; (d) the lack of relationship between the analgesic and antidepressant-like effects of examined antidepressants compounds. © 1998 Elsevier Science Inc.

Antidepressants Analgesia Writhing test Porsolt test Rats

THE numerous anecdotal reports and more recent controlled clinical studies revealed the efficacy of antidepressant drugs in neurogenic pain syndromes (painful diabetic neuropathy, postherpetic neuralgia, headaches, facial pain, central pain), in rheumatological disorders and in cancer pain [reviews: (25,27, 32,33,44,52,53); meta-analysis: (35)]. Analgesic properties of antidepressant drugs have also been reported in different experimental tests of nociceptive activity performed on healthy volunteers under controlled laboratory conditions (15,18,39) These clinical studies in humans indicate intrinsic analgesic activity of antidepressants independent from their psychotropic effect. Such conclusions is substantiated by following findings: a significant pain relief in nondepressed patients; an analgesic effect in healthy volunteers in acute experiments; a faster onset of analgesic than antidepressant effect; the analgesic doses lower than those used for the treatment of depression.

Some results from experimental studies on animals confirm that antidepressant drugs may have analgesic properties. The majority of animal studies were performed with single-dose administration of agents in acute models of pain reaction: the electrical stimulation of the foot (11) and the tail (23,34, 43,54), the hot-plate test (1,6,21,22,28,30,34,51), the tail flick test (1,20,21,28,34), the mechanical pressure of the tail (41), and the paw (3,5,9). A potent antinociceptive effect was also demonstrated in chemical tests of subchronic pain: in the formalin test (2,54) and in the writhing test (6,28,34,45,46,48). However, some authors reported also negative findings in this respect (12,14,49,50).

Although antidepressants are used in the management of pain, the site and the mechanism of their analgesic action remains unclear. The available experimental data are often contradictory and do not allow to draw any unequivocal conclu-

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sion. The modulation of nociception by antidepressants is probably centrally mediated, but the involvement of spinal or/and supraspinal structures is at present not well established. The most popular hypothesis has suggested the involvement of serotonergic (20,23,46,51,54), noradrenergic (2,46,54), and opioidergic systems in the changes of pain threshold caused by antidepressants. The involvement of central serotonin pathways in the modulation of pain has been known for many years (7,8,55), and most pharmacological data so far revealed that facilitation of central serotonergic transmission is potentially antinociceptive, whereas inhibition of serotonergic activity increases the sensitivity to noxious stimuli. There are also many experimental data supporting the involvement of noradrenergic pathways in the control of nociception. In general, stimulation of noradrenergic system produces antinociceptive effect, whereas its inhibition decreases pain threshold to various types of noxious stimuli (31,40). These facts, along with well-documented blocking action of antidepressants on the reuptake of monoamines, strongly suggest the monoamine-related mechanism of analgesic effect induced by these drugs. Moreover, it has appeared recently that some opioid analgesics, in addition to their action at opioid receptors, inhibit at relevant concentrations the uptake of serotonin and noradrenaline, and this mechanism may enhance analgesic activity of these compounds (17). For example, tramadol is a well-known synthetic analgesic considered to be the μ receptors agonist and inhibitor of monoamine reuptake. On the other hand, naloxone, an opioid receptor antagonist, was found to inhibit antinociceptive effect of antidepressant drugs (2,11, 20, 24,30,41,54).

Having in mind all the above facts and considerations, we have decided to reevaluate the problem of an involvement of antidepressants in pain perception. One of the main objectives of this study was to investigate in one laboratory, in a wide battery of tests, the effects of antidepressants with different biochemical mechanism of action belonging to different chemical categories. The following questions were put: (a) is there any test specificity for the analgesic effects of antidepressants? (b) Do the different antidepressants have similar profile of action in the models of pain reaction? (c) Does the analgesic effect of antidepressants change in the course of repeated administration? (d) Is there any correlation between the analgesic and antidepressant-like activity of antidepressants, and between their behavioral effects and blood and brain concentrations? For that purposes we tested four antidepressant drugs with different biochemical profiles of action, in four analgesic tests, in the open field and in the Porsolt tests, after single and chronic (21 days) drug administration. Additionally, determination of blood and brain concentrations of imipramine using fluorescence polarization immunoassay was performed.

METHOD

Animals

Male Wistar rats, bought from a licensed breeder, weighing 200 ± 20 g at the beginning, and 350 ± 30 g at the end of the experiment, were used. Animals were housed five to a cage (40 × 30 × 20 cm) under a 12 L:12 D cycle (lights on at 0600 h) and at constant temperature (21°C) in a ventilated room with standard laboratory food and tap water available ad lib. The only exception was a group of animals used in the writhing test, deprived of food 24 h before experimental session (see Writhing test).

General Procedure

Drugs were administered intraperitoneally at the dose of 5, 10, or 25 mg/kg. Analgesia was measured by four methods: the chemical writhing test with 2% acetic acid, the mechanical tail withdrawal test, the thermal tail flick test, and the electrical flinch-jump test. The effect of drugs on forced swimming and motor activity was evaluated in the Porsolt and open-field tests, respectively. The experiments, with exception of the writhing test, were performed 30 min after drugs injection and consisted of a pain threshold measurement (one animal–one test); a 5-min observation in the open-field test; a 5-min interval with rats remaining in their home cages; a 5-min testing in the Porsolt test. Then the rats received repeated drugs injections (IP once a day for 21 days) and the procedure described above was repeated 30 min after 21st injection. Thus, separate groups of animals were subjected to one analgesic test only on two occasions: after the 1st and 21st drug injection. The other groups of animals were tested in the writhing test 30 min after the first or last chronic drugs administration. Each animal received only one injection of acetic acid solution without any other behavioral testing. Each experimental group consisted of 7–10 animals. Additionally, changes in the blood and brain concentrations of imipramine were analyzed in another group of animals not tested behaviorally.

Drugs

The following drugs were used: imipramine hydrochloride (Polfa-Starogard, PL), amitriptyline hydrochloride (Polfa-Kraków, PL), citalopram, a selective serotonin reuptake inhibitor (H. Lundbeck A/S, Denmark), and maprotiline hydrochloride, a selective noradrenaline reuptake inhibitor (Ciba-Geigy, CH). All drugs were dissolved in water for injection and administered IP in a volume of 2 ml/kg of body weight or PO in a volume of 5 ml/kg of body weight. The doses of drugs were selected on the basis of our own previous experience (19), and literature data (54).

Analgesic Tests

Writhing test. The writhing test was used to measure changes in nociceptive threshold to the chemical stimulus. Each animal, deprived of food 24 h before the experimental session, was injected IP with 0.5 ml of a 2% aqueous solution of acetic acid and placed in a individual container (40 × 30 × 20 cm) for observation. The number of writhes was counted during a 60-min observation period. A writhe was defined as stretching of the hind limbs accompanied by a contraction of the abdominal muscles. The injection of 2% acetic acid caused self-limiting reaction, which disappeared without any visible damage to animal within an hour after injection.

Tail-withdrawal test. The tail-withdrawal test was used to measure changes in the nociceptive threshold to the tactile stimulus. The stimulus was applied with the analgesymeter (Ugo-Basile, Italy), which generated a linearly increasing mechanical force, applied by a conical piece of plastic with a dome-shaped tip about 1.5 cm from the tail tip. During measurement animals were briefly restrained with experimenter hand by gently wrapping them in wood-wool pieces. The results represent the maximal pressure (expressed in arbitrary units, 1 unit = 30 g) tolerated by the animal. The mean of three consecutive values (separated by intervals of 10 s) was determined. To avoid tail damage the cutoff = 750 g was established.

Tail-flick test. The tail-flick test was used to measure changes in the nociceptive threshold to the thermal stimulus. The stimulus was applied with a Tail flick Unit (Ugo-Basile, Italy), which generated a beam of light 55°C focused by an embodied parabolic mirror about 10 cm from the tail tip. During measurement animals were briefly restrained by gently wrapping them in wood-wool pieces. The results represent the mean latency of moving away the tail (in seconds), taken from three consecutive trials (separated by intervals of 10 s). To avoid tail damage the cutoff = 10 s was established.

Flinch-jump test. The flinch-jump test was used to measure changes in nociceptive threshold to the electrical stimulus. Animals were tested in a Plexiglas chamber with a floor (25 × 25 cm) composed of 16 bars, through which scrambled electric foot shocks were delivered by shock generator. Each trial, after a 2-min long period of adaptation to the chamber, began with animals receiving a 4-ms foot shock at a current intensity set on 0.1 mA. Subsequent shocks were increased in equal 0.05-mA steps at 10-s intervals. The results represent the flinch and the jump thresholds in mA. The flinch threshold was defined as the lowest shock intensity that elicited any detectable response. The jump threshold was defined as the lowest shock intensity that elicited simultaneous removal of at least three paws (both hindpaws) from the grid. To avoid foot damage the cutoff = 2.0 mA was established.

Porsolt test. The Porsolt test was used to measure changes in the behavioral despair reaction of animals. Rats were tested in glass cylinders (height = 40 cm, diameter = 18 cm) containing 18–20 cm of water (temperature = 25 ± 1°C). Twenty-four hours before the experimental session a pretest was performed without administering drugs. Animals were individually put into the water and forced to swim for 15 min. During the experimental session animals were replaced into the cylinders and the total activity time was measured for 5 min. The results represent the time of activity of rats (swimming, diving, jumping, struggling, forepaw treading) in seconds.

Open-field test. Open field (80 × 80 × 30 cm square box made of wood) testing was performed in a soundproof room under dim light (60 W lamp situated 1.5 m above the center of the testing area) and white noise condition (65–75 dB) without previous adaptation. The rats were observed via closed-circuit television. Each rat was gently placed into the center of the box and the number of ambulations (the parameter defined as a movement of an animal through white lines dividing black floor) was counted for 5 min, and taken as a measure of locomotor activity.

Biochemical Analysis

Imipramine and its desmethyleted metabolites were assayed in the blood and in the brain of a separate group of animals with help of a fluorescence polarization immunoassay using Abbott TDx analyzer. The method combines two techniques: competitive protein binding and fluorescence polarization (47). The schedule of drug administration and the time of biochemical analysis were exactly the same as in the case of behavioral experiments (see General Procedure). Rats were killed by decapitation 30 min after single or final imipramine injection. The samples of trunk blood collected, and the brains were stored frozen at −20°C.

Data Analysis

One-way analysis of variance ANOVA was used for comparisons of the data and Newman–Kuels test was made as post hoc. Where appropriate, the significance of differences between means was determined also by Student's two-tailed *t*-test. A p-value less than 0.05 was considered significant.

RESULTS

Writhing Test (Table 1)

Single-dose administration. One-way ANOVA revealed a significant overall effect of the treatment with imipramine, $F(3, 28) = 14.77$, $p < 0.00001$; amitriptyline, $F(3, 28) = 10.04$, $p < 0.001$; citalopram, $F(2, 21) = 8.31$, $p < 0.01$; and maprotiline, $F(3, 28) = 5.77$, $p < 0.01$. Post hoc comparisons of means (Newman–Keuls test) showed dose-related analgesic effect of all examined doses of imipramine and amitriptyline, and of higher doses of citalopram and maprotiline. Imipramine and amitriptyline, at the dose of 25 mg/kg, completely blocked pain-induced behavior.

Chronic administration. One-way ANOVA showed a main effect of the treatment with imipramine, $F(2, 21) = 6.93$, $p < 0.01$; amitriptyline, $F(2, 21) = 7.70$, $p < 0.01$; and maprotiline, $F(2, 20) = 7.67$, $p < 0.01$]; but not with citalopram, $F(2, 21) = 3.07$, $p = 0.07$. Post hoc comparisons (Newman–Keuls test) revealed that both examined doses (5 and 10 mg/kg) of imipramine, amitriptyline, and maprotiline caused potent analgesic effect. No significant antinociceptive responses, in spite of some tendency, were observed in the groups of animals treated with citalopram at the doses of 5 and 10 mg/kg.

Writhing Test (Table 2)

Single-dose PO administration. One-way ANOVA revealed a significant effect of the treatment with imipramine at the dose of 10, $F(2, 17) = 6.46$, $p < 0.01$, and 25 mg/kg, $F(2, 18) = 11.89$, $p < 0.001$, maprotiline at the dose of 10 mg/kg, $F(2, 17) = 5.08$, $p < 0.05$, but not of citalopram. Imipramine significantly reduced the number of writhes in both doses, and appeared more effective in the test performed 60 min after drug administration. Maprotiline has similar analgesic po-

TABLE 1

ANTINOCICEPTIVE ACTIVITY OF ANTIDEPRESSANT DRUGS AFTER SINGLE (1) AND 21 DAYS OF CHRONIC (21) IP ADMINISTRATION IN THE RAT WRITHING TEST

		Writhing Test			
			1		21
Drug	Dose (mg/kg)	*n*	Number of Writhes	*n*	Number of Writhes
Vehicle		8	31 ± 6	8	40 ± 12
Imipramine	5	8	13 ± 2†	8	12 ± 5*
	10	8	7 ± 2‡	8	2 ± 1†
	25	8	0 ± 0‡		(—)
Amitriptyline	5	8	14 ± 3*	8	8 ± 3†
	10	8	6 ± 3†	8	3 ± 1†
	25	8	0 ± 0‡		(—)
Citalopram	5	8	20 ± 7	8	16 ± 5
	10	8	9 ± 4†	8	15 ± 5
	25	8	4 ± 3†		(—)
Maprotiline	5	8	16 ± 28	8	3 ± 1†
	10	8	7 ± 2†	8	5 ± 4†
	25	8	2 ± 2†		(—)

The data are shown as means ± SEM. *n* = number of rats. *$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ vs. control. (—) not studied.

TABLE 2

ANTINOCICEPTIVE ACTIVITY OF ANTIDEPRESSANT DRUGS ADMINISTERED PO IN THE RAT WRITHING TEST

		Writhing Test				
Drug (Dose (mg/kg))		Vehicle	Imipramine 10	Imipramine 25	Maprotiline 10	Citalopram 25
Number of writhes	30 min	40 ± 7	25 ± 8*	15 ± 3*	17 ± 5*	17 ± 7
	60 min		12 ± 5†	1 ± 1‡	20 ± 8*	25 ± 10

The data are shown as means ± SEM. The drugs were given either 30 or 60 min prior to an irritant injection, and rats' behavior was scored as described in the method. *$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$. Number of rats in each group = 7.

tency 30 and 60 min after administration. On the other hand, the influence of citalopram, in spite of a clear-cut tendency, did not reach the statistically significant level.

Tail Withdrawal Test (Table 3)

Single-dose administration. One-way ANOVA revealed an overall significant effect of the treatment with citalopram, $F(2, 21) = 3.77$, $p < 0.05$, and maprotiline, $F(2, 21) = 10.33$, $p < 0.001$. Post hoc comparisons of means (Newman–Keuls test) showed that all examined doses of maprotiline attenuated the influence of the nociceptive stimulus. Citalopram was active at the dose of 10 mg/kg only. No significant antinociceptive effects of imipramine and amitriptyline, at least at the tested dose range, were observed.

Chronic administration. No significant antinociceptive responses were noted after chronic administration of imipramine, amitriptyline, citalopram, and maprotiline.

Tail Flick and Flinch-Jump Test

Amitriptiline, imipramine, maprotiline, and citalopram administered intraperitoneally at the same dose range and according to the same experimental protocol as in the tail withdrawal test, did not modify in a significant way the tail-flick and the flinch-jump reactions. Moreover, the drugs given either on an acute or chronic basis did not show any tendency to change animals behavior controlled by pain (data not shown). The only exception was the highest dose of maprotiline (25 mg/kg), being marginally (but statistically significant) effective after single administration, in both behavioral models.

Porsolt test (Table 4)

Single-dose administration. One-way ANOVA revealed an overall significant effect of the treatment with maprotiline only, $F(2, 21) = 10.44$, $p < 0.001$. Post hoc comparisons of means (Newman–Keuls test) showed that only the highest examined dose of maprotiline and imipramine significantly shorted the duration of immobility of rats. Moreover, in the case of citalopram, there was a tendency for the drug to increase the total duration of immobility.

Chronic administration. One-way ANOVA revealed an overall significant effect of the treatment with imipramine, $F(2, 22) = 10.98$, $p < 0.001$, and amitriptyline, $F(2, 17) =$

TABLE 3

ANTINOCICEPTIVE ACTIVITY OF ANTIDEPRESSANT DRUGS AFTER SINGLE (1) AND 21 DAYS OF CHRONIC (21) IP ADMINISTRATION IN THE RAT TAIL WITHDRAWAL TEST

		Tail Withdrawal Test			
Drug	Dose (mg/kg)	*n*	1 Tolerated Pressure (Arbitrary Units)	*n*	21 Tolerated Pressure (Arbitrary Units)
Vehicle		10	8.5 ± 0.9	10	11.0 ± 1.3
Imipramine	5	10	11.0 ± 1.3	10	13.4 ± 1.3
	10	10	12.7 ± 1.5	10	10.0 ± 1.0
	25	8	11.5 ± 1.3	8	12. ± 2.6
Amitriptyline	5	10	8.7 ± 0.9	10	14.3 ± 1.6
	10	7	9.9 ± 1.7	7	11.8 ± 1.6
	25	7	9.2 ± 1.2	7	11.1 ± 2.6
Citalopram	10	8	12.9 ± 1.3*	8	11.7 ± 1.8
	25	8	13.1 ± 2.1	8	11.3 ± 1.1
Maprotiline	5	10	13 ± 1.5	10	14.7 ± 2.1
	10	8	15.5 ± 1.6†	8	12.3 ± 2.0
	25	8	16.8 ± 2.0†		(—)

The data are shown as means ± SEM. *n* = number of rats. *$p < 0.05$, †$p < 0.01$ vs. controls. (—) not studied.

TABLE 4

THE EFFECT OF ANTIDEPRESSANT DRUGS AFTER SINGLE (1) AND 21 DAYS OF CHRONIC (21) ADMINISTRATION ON SWIMMING DURATION IN RAT PORSOLT TEST

		Porsolt Test			
Drug	Dose (mg/kg)	*n*	1 Swimming Duration (s)	*n*	21 Swimming Duration (s)
Vehicle		10	85 ± 14	10	63 ± 17
Imipramine	5	10	114 ± 17	10	116 ± 23*
	10	10	137 ± 24	10	191 ± 21‡
	25	10	167 ± 24*	8	208 ± 26†
Amitriptyline	5	10	111 ± 16	10	116 ± 13†
	10	7	132 ± 9	7	164 ± 20†
	25	7	163 ± 23	7	222 ± 18‡
Citalopram	10	8	44 ± 13	8	24 ± 8
	25	8	40 ± 13	8	38 ± 11
Maprotiline	5	10	102 ± 11	10	53 ± 11
	10	8	76 ± 13	8	93 ± 33
	25	8	163 ± 15‡	(—)	(—)

The data are shown as means ± SEM. *n* = number of rats. *$p <$ 0.05, †$p < 0.01$, ‡$p < 0.001$ vs. controls. (—) not studied.

TABLE 5

THE EFFECT OF ANTIDEPRESSANT DRUGS AFTER SINGLE (1) AND 21 DAYS OF CHRONIC (21) ADMINISTRATION ON RAT LOCOMOTOR ACTIVITY IN THE OPEN FIELD TEST

		Open-Field Test			
Drug	Dose (mg/kg)	*n*	1 Locomotor Activity	*n*	21 Locomotor Activity
Vehicle		10	30 ± 6	10	25 ± 8
Imipramine	5	10	25 ± 5	10	10 ± 3
	10	10	20 ± 4*	10	9 ± 2†
	25	10	21 ± 5*	8	25 ± 7
Amitriptyline	5	10	28 ± 5	10	16 ± 5
	10	7	26 ± 8	7	20 ± 5
	25	7	12 ± 4*	7	26 ± 9
Citalopram	10	8	27 ± 4	8	18 ± 4
	25	8	25 ± 4	8	27 ± 7
Maprotiline	5	10	21 ± 5	10	19 ± 6
	10	8	12 ± 2†	8	9 ± 3*
	25	8	5 ± 2‡	(—)	(—)

The data are shown as means ± SEM. *n* = number of rats. *$p <$ 0.05, †$p < 0.01$, ‡$p < 0.001$ vs. controls. (—) not studied.

12.64, $p < 0.001$. Post hoc comparisons of means (Newman–Keuls test) showed that all examined doses of both drugs caused statistically significant antidespair effects. No significant changes in rat behavior were observed after administration of citalopram and maprotiline at the examined doses. Citalopram caused, although in a not significant way, some inhibition of animals active behavior. On the other hand, maprotiline (10 mg/kg) increased almost by 100% duration of rat activity in the test; however, due to high variability of individual data this effect was not statistically significant.

Open-Field Test (Table 5)

Among all examined drugs, only single-dose administration of imipramine, $F(2, 25) = 4.03$, $p < 0.05$, and maprotiline, $F(2, 21) = 12.92$, $p < 0.001$ significantly inhibited rats motor activity. Decrease in motor activity was more potent after acute drug administration, and was characterized by high variability of behavioral data. The behavior of rats treated with amitriptyline and citalopram was not changed in a significant way.

Biochemical Analysis (Table 6)

The concentrations of imipramine and its desmethylated metabolites differed significantly in the brain and in the blood in the way dependent on the dose and the time of drug administration. The level of tricyclic compounds appeared higher in the brain than in the blood both after single-dose and chronic administration of the drug. Cmax after chronic imipramine treatment at the dose of 5 mg remained rather stable in the blood and brain, in comparison with single dose administration. On the other hand, much higher tissue concentration of imipramine given repeatedly at the dose of 10 mg/kg were found in comparison with an acute experiment. However, the brain to blood ratio of imipramine concentration (three to four) remained stable over the time and the dose of the drug administered.

DISCUSSION

The antidepressant-induced analgesia appeared to be test dependent and most potent in the rat writhing test. In other tests using thermal, mechanical, and electrical stimuli antidepressants did not significantly change the pain threshold, with only some exceptions. The test dependency of the analgesic effect of antidepressants is repeatedly found, and chemical tests belong to the most sensitive. Spiegel et al. (48) showed that different tricyclic antidepressant drugs were active in the mouse writhing model of nociception with ED_{50} of about 2.0–3.0 mg /kg IP, whereas the same compounds administered at much higher dose range appeared ineffective in the tail-flick test. Also, Fialip et al. (28) found that metapramine potently inhibited writhing reactions in the phenylbenzoquinone test in mice (ED_{50} = 9.9 mg/kg IP), but it revealed only weak activity in the tail-flick test, with the highest dose of 20 mg/kg IP being only marginally active. Furthermore, tricyclic antidepressants expressed much more potent antinociceptive activity in the formalin than in the tail electric stimulation test (54). The reasons for differences in the sensitivity of animal models to antidepressants are not clear. It is conceivable, that the test dependency of antidepressant actions most probably reflects different participation of peripherally and/or centrally located mechanisms, in distinctly evoked nociceptive reactions. For example, the tail-flick and the tail-withdrawal tests involve single monosynaptic spinal reflexes, whereas in the writhing and the flinch-jump tests contribution of more complex and centrally located mechanisms is suggested. The present data indicate superior activity of antidepressants in a model of a subchronic pain. This finding is supported also by some clinical observations (see the introductory paragraphs).

The lack of correlation between the effect of antidepressants in the writhing and open-field tests demonstrated that modification of animal behavior induced by pain were not due to nonspecific changes in rats gross behavior. For example, imipramine and amitriptyline revealed analgesic activity at

TABLE 6

BLOOD AND BRAIN CONCENTRATIONS OF IMIPRAMINE AFTER SINGLE (1) AND CHRONIC (21) DRUG ADMINISTRATION

		1		21	
Drug	Dose (mg/kg)	Blood Concentration μg/ml	Brain Concentration μg/g	Blood Concentration μg/ml	Brain Concentration μg/g
Imipramine	5	203.3 ± 19.3	548.4 ± 101.5*	206.6 ± 13.9	785.3 ± 125.3†
	10	462 ± 55.9	1658 ± 160.4†	794.2 ± 60.8‡	3167 ± 193.9†‡

*$p < 0.01$, †$p < 0.001$ blood vs. brain in appropriate groups. ‡$p < 0.001$ acute vs. chronic administration in appropriate tissues. Number of rats in each group = 10.

the doses not inducing any detectable motor impairment. Accordingly, it was shown by others that antidepressants with noradrenergic profile of action increased pain threshold without producing changes in the open-field behavior, in rats (34). The part of the experiment with intragastric drugs administration pointed out that the activity of antidepressants in the writhing test did not depend on their interaction with local, peritoneally situated, mechanisms of nociceptive stimulus perception and/or transmission. All tested drugs, with exception of citalopram, when given by a gavage-produced potent analgesic effect of similar magnitude to that after intraperitoneal administration. The present data do not allow to conclude about the site of analgesic action of antidepressants. However, the hypothesis of the central location of their antinociceptive effect may be suggested. Intraperitoneal injection of an acetic acid solution is known to cause a biphasic reaction, with the short latency immediate response most probably reflecting central mechanism of pain perception. It is followed by a hyperalgesic response representing adaptative changes in pain sensitivity, secondary to inflammatory processes (29). Although antidepressants may have some antiinflammatory properties due to their action on prostaglandin synthesis, depression of SP release from peripheral nerve endings, and an interference with the function of immune cells (10), it was previously shown that these drugs differently affect inflammation and pain (3,9). For example, whereas clomipramine significantly reduced the oedema and the hyperalgesia induced by yeast injection in the paw, the intensity and time course of these two effects differed (9). Additionally, adrenalectomy enhanced the antinociceptive but not the antioedema action of clomipramine. Thus, the data suggest the dissociation of the analgesic and antiinflammatory effects of a single injection of clomipramine.

Secondly, intracerebroventricular microinjections of antidepressant drugs were shown to evoke strong analgesic effect in the writhing test [(46,48); our preliminary data]. Furthermore, reported in this paper significantly higher concentrations of a tricyclic drug in the brain after peripheral administration also indirectly points at the central origin of its antinociceptive activity.

Although the present results do not allow to discuss in detail the mechanisms of analgesic action of examined drugs, it can be inferred that both noradrenergic and serotonergic systems may play a role. Maprotiline acts selectively on noradrenergic transmission at the central adrenergic synapses via blockade of the reuptake of noradrenaline at the nerve endings about 470 times more potent that reuptake of serotonin (42). Imipramine and amitriptyline are much less selective in this respect, and the action of citalopram is presumed to be exclusively bound up with inhibition of the neuronal uptake of serotonin (42). Tricyclic drugs nonselectively blocking reuptake of monoamines appeared to express the most potent analgesic effect, completely antagonizing the behavioral reactions induced by the nociceptive stimulus in the writhing test. This finding is consistent with other authors results (4), and clinical observations (26). The comparison of analgesic potencies of maprotiline and citalopram indicates a stronger effect of noradrenaline uptake blocker. Accordingly, it appeared that 63% of animal studies on nociception reported negative results with selective serotonin reuptake inhibitors, and 64% of them showed positive results with antidepressant drugs inhibiting noradrenaline uptake (26).

After chronic drugs administration there appeared a tendency for analgesic effects to be either diminished (citalopram in the writhing test, and maprotiline in the tail-withdrawal and flinch-jump tests), or not changed. Although the majority of authors observed a long-lasting analgesia accompanying chronic treatment with antidepressants (2,4,16,22,24,28), some of them found this effect to be limited (52). It is noteworthy, that in the clinic these drugs are used repeatedly for long periods of time, in chronic pain syndromes [reviews: (25,27,33,44, 52,53); meta-analysis: (35)]. These results contrast with biochemical data on changes in the blood and brain concentrations of imipramine. The brain level of this tricyclic drug administered at the dose of 10 mg/kg, was almost doubled in the course of a 3-week-long treatment. Moreover, there appeared more than 400% increase in imipramine brain concentration after the dose of 10 mg/kg, in comparison with the dose of 5 mg/kg, in chronically treated group. The same refers to the acutely injected rats. Thus, it seems that there is no direct correlation between the blood or brain concentration of imipramine and its behavioral effect in the writhing test, and changes in the pharmacokinetic processes do not play a role.

The writhing test data contrast also with the Porsolt test results. It appeared that the weak antiimmobility influence of tricyclic antidepressants after acute treatment was potently and significantly increased by chronic drug administration. The antiimmobility effect of antidepressant drugs is a very well-known phenomenon, probably reflecting their psychotropic action (37,38). Thus, the different dose range and latencies of analgesic and antidepressant-like effects of antidepressants seem to indicate, that there is no unequivocal and synchronized in time correlation between their psychotropic and analgesic activity. Such corollary is congruent with some clinical data pointing at significant pain relief in nondepressed patients, and faster onset of analgesic than antidepressant effect of antidepressant therapy (see the introductory paragraphs). It is also noteworthy that citalopram was not active in

the Porsolt test, over all examined doses. This result confirms other authors' findings, and implies that serotonergic system is not important for expression of rat behavior in this test (36). For example, Borsini in his recent review article, concludes that serotonin uptake inhibitors are devoid of any activity in rats, and induce an antiimmobility effect in mice (13). Such conclusion accords with our own previously published experimental data (19). Surprisingly, maprotiline appeared also inactive in the Porsolt test across all examined doses and treatments, except for the highest dose of 25 mg/kg. Although high variability of individual data may be one of the reasons, these results can be considered as further underlining the predictive validity of this test, as tricyclic antidepressants are believed to be more efficacious than maprotiline in the treatment of endogenous depression. Apparently, besides noradrenaline, other neurotransmitters play a role in producing antidepressant effect. Another point deserving mentioning is that in contrary to the antinociceptive activity, there may be some correlation between the brain concentration of imipramine, and its behavioral effects in the Porsolt test. Accordingly, after chronic drug administration there appeared in a parallel way a significant enhancement of the blood and brain imipramine concentrations and rat behaviour in the Porsolt test. Thus, the pharmacokinetic explanation of this part behavioral data cannot be neglected.

In sum, it appears that whereas tricyclic drugs seem to play important role both, in the antinociception and in Porsolt test, their behavioral effects apparently do not overlap. The writhing test modeling subchronic pain was also proved to be most sensitive to the analgesic action of antidepressant drugs. The antidepressants-induced analgesia was a short-latency, and long-lasting phenomenon, most probably of central origin but independent from changes in their psychotropic activity. Apparently, more research is needed, with central administration of drugs to solve the problem of a mechanism of antinociceptive activity of antidepressant drugs.

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