Pharmacology Biochemistry and Behavior, Vol. 64, No. 3, pp. 541–548, 1999

0091-3057/99/$–see front matter

ELSEVIER

PII S0091-3057(99)00102-1

Dopaminergic and Opioidergic Mediations of Tricyclic Antidepressants in the Learned Helplessness Paradigm

A. BESSON,* A. M. PRIVAT,* A. ESCHALIER† AND J. FIALIP*

*Equipe NPPUA (NeuroPsychoPharmacologie, Université d'Auvergne), *Laboratoire de Pharmacologie, Faculté de Pharmacie, †Laboratoire de Pharmacologie Médicale Faculté de Médecine, 28, place Henri-Dunant, BP 38, 63001 Clermont Ferrand Cedex 1, France*

Received 25 June 1998; Revised 12 April 1999; Accepted 12 April 1999

BESSON, A., A. M. PRIVAT, A. ESCHALIER AND J. FIALIP. *Dopaminergic and opioidergic mediations of tricyclic antidepressants in the learned helplessness paradigm.* PHARMACOL BIOCHEM BEHAV **64**(3) 541–548, 1999.—The roles of dopaminergic and opioid neurotransmissions in the activity of three tricyclic antidepressants endowed with different monoamine-reuptake properties [desipramine (DESI), imipramine (IMI), amineptine (AMN)] were examined using a behavioral model of depression in rats; the learned helplessness paradigm. In this model, exposure of rats to inescapable shocks (day 1) produced a subsequent escape deficit in a shuttle box test (days 3, 4, and 5). The escape deficit was reversed by AMN, DESI, and IMI administered twice daily for 5 days (16 and 32 mg/kg/day, $p < 0.05$, days 3, 4, and 5). In addition, AMN tended to enhance the motor activity of rats during the intertrial intervals, but on the first shuttle-box test only (day 3: $p < 0.05$, control vs AMN). Haloperidol, a preferential D_2 dopamine receptor antagonist, acutely injected IP (37.5 μg/kg), suppressed the behavioral activity of DESI and IMI but not that of AMN. Naloxone, a preferential mu-opioid receptor antagonist, acutely injected IP (0.5 mg/kg), suppressed the behavioral activity of IMI but not that of DESI and AMN. It is concluded that an increased dopaminergic activity is a neurochemical effect common to the different tricyclic antidepressants (via a presynaptic mechanism for AMN and a postsynaptic mechanism for DESI and IMI), whereas an increased mu-opioid neurotransmission does not appear to be essential. © 1999 Elsevier Science Inc.

Antidepressants Haloperidol Dopamine Opioid Rat Learned helplessness

THE learned helplessness paradigm meets the different criteria of validity (face, predictive, construct validities) to qualify as a reliable animal model of depression [for review, see (24,37,38,69)]. In this model, previous exposure of rats to an uncontrollable aversive event induces a subsequent impairment in the performance of an escape task (37,49,69). This behavioral alteration is reversed by the subchronic administration of various antidepressant drugs (8,20,43–45,56,57). Hence, this model appears suitable to study the neurobiological mechanisms of action of antidepressant drugs.

Preclinical studies have focused mainly on noradrenaline and serotonin neurotransmissions in the mechanism of action of antidepressants (6,12,19,26,31,51). However, there is increasing evidence that the biochemical effects of these drugs are much more complex, involving other neuromediators, among which dopamine (DA) may play a crucial role. For instance, in vivo binding experiments have indicated that repeated administration of amitriptyline, imipramine, and mianserine increased the affinity of a specific D_2 DA ligand in the striatum and limbic forebrain and the density of D_2 DA receptors in the limbic region only (40). In addition, various tricyclic antidepressants enhance DA release in the prefrontal cortex and the striatum (9,27,64,65). Fluoxetine, a specific serotonin reuptake inhibitor, also appears to interact with DA systems, with effects depending on the administration schedule and the brain area studied [see (27,40,64,65), for review]. Other microdialysis studies have shown that long-term administration of desipramine produced an increase in the ability of amphetamine to release DA in the nucleus accumbens (NAC) (47) as well as in the ventral tegmental area (VTA) (61),

Requests for reprints should be addressed to Prof. J. Fialip, Laboratoire de Pharmacologie, Faculté de Pharmacie, 28, place Henri-Dunant, BP 38, 63001 Clermont Ferrand Cedex 1, France.

whereas short-term treatment did not (61). From a behavioral point of view, long-term treatment with various antidepressants increases the locomotor effects of indirect and direct dopamine stimulant drugs, for example, amphetamine, apomorphine, and quinpirole, suggesting a supersensitivity of postsynaptic D_2 DA receptors induced by chronic antidepressant treatments (13,39,50,59). In two animal models of depression, the forced swimming test and stress-induced anhedonia, the effects of various antidepressants have been found to be abolished by D_2 DA receptor antagonists (11,52,70), while D_2/D_3 DA receptor agonists presented antidepressant-like effects (46,70).

An opioid mediation has also been suggested in the mechanism of action of antidepressant drugs. Few behavioral studies are available, but they support the hypothesis of an opioid mediation in the thymoanaleptic properties of antidepressants. Naloxone inhibits the effects of various tricyclic antidepressants in the forced swimming test, and the effect of imipramine in learned helplessness (15,42). Binding and biochemical studies are more controversial regarding the interaction of antidepressants with opioid systems, depending on the antidepressant treatment used and the brain area studied. For instance, a decrease, an increase, and no change in opioid binding by tricyclic antidepressants have all been reported (2,23,29,60). Repeated administration of amitriptyline has been found to enhance Met-enkephalin levels in the hypothalamus but not in the cerebral cortex , while amoxapine did not affect its levels in either region (23). In another biochemical study, acute or prolonged administration of IMI and single or repeated electroconvulsive shocks (ECS) were found to increase the levels of Met-enkephalin in the NAC, while only ECS induced increased levels of Met-enkephalin in the VTA (18). In addition, repeated IMI or ECS, but not their single administration, increased mRNA coding for proenkephalin in the NAC (18).

Interestingly, using the learned helplessness model in rats we found that haloperidol, a preferential D_2 DA receptor antagonist, and naloxone, an opioid antagonist, produced an escape deficit per se in rats not previously exposed to inescapable shocks, and that they both suppressed the antidepressant-like effects of morphine (3,4).

These results raised the question of whether increased DA and/or opioid neurotransmission can account for the reversal of escape deficit by antidepressant drugs in the learned helplessness model. In the present study, we studied the activity of amineptine (AMN), an antidepressant with DA-uptake inhibitory properties (7) and of two classical tricyclic antidepressants, desipramine (DESI) (noradrenaline reuptake inhibition) and imipramine (IMI) (serotonin and noradrenaline reuptake inhibition) (53,54), through their interaction with haloperidol and naloxone.

METHOD

Animals

Male Wistar rats (Janvier, France) weighing 195–220 g on the first day of the experiments were used. They were housed six to eight per cage under standard laboratory conditions, with free access to food and tap water. All the experiments started 1 week after reception of the rats.

Apparatus and Experimental Procedure

The experimental procedure used below refers to the method described by Martin et al. (43). The experimental protocol was approved by a local ethical committee and complied with the guidelines for animal experimentation of the National Institutes of Health (1985) and with the relevant French legislation (1987 and 1988).

Shock pretraining. The rats were divided into two groups. The first group (stressed, S) was exposed to unsignalled inescapable electric footshocks (IS) delivered through a stainless steel grid floor (1 × 0.3 m) allowing simultaneous stimulation of several rats. Randomized scrambled shocks (15 s duration, 0.8 mA, every min ± 15 s) were generated by an Apelex model "LE 100-26" shocker connected to an Apelex "LI 10-31S" random generator. Stressed rats subjected to the IS procedure were individually placed in a plexiglas chamber (20 × 10 × 10 cm) on the electrified grid floor for 1 h. A second group (nonstressed, NS) was placed for 1 h in the same conditions, except that the shock generator was turned off. Pretraining was performed on day 1, between 0800 and 1500 h.

Avoidance-escape training. Forty-eight hours after pretraining (i.e., day 3), all the rats were exposed to an avoidance-escape task in automated two-way shuttle-boxes (OSYS-Orga System) with black walls and an electrified grid floor. The rats were placed singly in one of the four shuttle-boxes. A 5-min environmental adaptation period was allowed before the beginning of 30 trials (intertrial interval = 24 s). For each trial, a light signal came on for the first 3 s (conditioning signal), during which time the rats were allowed to avoid the shock (avoidance response). If no crossing occurred within this period, an electric footshock (3-s duration, 0.8 mA) was delivered. A single crossing from the electrified compartment to the other one made within this latter period was called an escape response. If no escape response occurred, light and shock were turned off, and there was an escape failure (learned helplessness behavior). The shuttle-box test was repeated on days 4 and 5, but no period of adaptation was used. Two parameters were recording in each shuttle-box session: escape failures (EF_s) and intertrial crossings (ITC_s). ITC corresponds to the crossing of the rat from one compartment to the other during the intertrial interval, and was considered as a measure of unconditioned motor activity (20).

Drug Administration

Antidepressant treatments. The administration schedule used refers to that described by Martin et al. (43). Only stressed rats were injected with drugs according to the protocol of Martin et al. (43) and to data appearing in the review of Thiebot et al. (68). Unstressed rats received vehicle. Antidepressants were injected repeatedly on 5 consecutive days. The first injection was administered 4 h after the shock pretraining on day 1, and then twice daily, in the morning (30 min before the escape test) and the evening (1800 h, except on day 5). Three doses were tested for each antidepressant (8, 16, and 32 mg/kg/day; 10 to 14 rats/dose) to determine the doses to be used in the interaction studies. These doses did not induce any signs of toxicity. The weight gain of treated rats was similar to that of untreated rats from day 1 to day 5, and there was no death up to 10 days beyond the end of the experiment. The first injection corresponded to the complete dose, and then half the dose was given at each injection. Antidepressants were injected intraperitoneally in a volume of 5 ml/kg of body weight. Imipramine hydrochloride (Ciba-Geigy, Rueil-Malmaison, France) and desipramine hydrochloride (Ciba-Geigy, Rueil-Malmaison, France) were dissolved in distilled water. Amineptine hydrochloride (Servier, Neuilly sur Seine, France) was dissolved in distilled water containing a few drops of dim-

ethylsulfoxide [D.M.S.O. (0.0001%)]. Each drug was studied separately.

Involvement of a dopaminergic mediation in the effects of antidepressant treatments. In this series of experiments, we chose a dose of antidepressant (32 mg/kg/day) that produced a complete reversal of the escape deficit in stressed rats, and a dose of haloperidol that did not induce an escape deficit by itself (37.5 μg/kg) (4). Haloperidol was administered intraperitoneally once a day, on days 3, 4, and 5, 15 min after the antidepressant and 15 min before the escape training.

Haloperidol (Janssen, Boulogne-Billancourt, France) was dissolved in distilled water and injected in a volume of 2.5 ml/kg of body weight.

Rats submitted to IS on day 1 were randomly divided into eight groups, comprising one control group (S-C), three antidepressant-treated groups (DESI, IMI, and AMN), one group given haloperidol alone (Hal), three groups given an acute dose of haloperidol in addition to one of the subchronic antidepressant treatments (DESI+Hal, IMI+Hal, AMN+Hal).

Rats not submitted to IS on day 1 were injected with distilled water (NS-C). At least 10 rats per group were used.

Involvement of an opioid mediation in the effects of antidepressant treatments. In this series of experiments, we chose a dose of antidepressant (32 mg/kg/day) that produced a complete reversal of the escape deficit in stressed rats, and a dose of naloxone that did not induce an escape deficit by itself (0.5 mg/kg) (3). Naloxone was administered intraperitoneally once a day, on days 3, 4, and 5, 15 min after the antidepressant and 15 min before the escape training.

Naloxone hydrochloride (Sigma, L'Isle-d'Abeau, France) was dissolved in distilled water and injected intraperitoneally in a volume of 5 ml/kg of body weight.

Rats submitted to IS on day 1 were randomly divided into eight groups,comprising one control group (S-C), three antidepressant-treated groups (DESI, IMI, and AMN), one group given naloxone alone (Nal), three groups given an acute dose of naloxone in addition to one of the subchronic antidepressant treatment (DESI+Nal, IMI+Nal, AMN+Nal).

Rats not submitted to IS on day 1 were injected with distilled water (NS-C). At least 10 rats per group were used.

Statistical Analyses

The number of EF_s and ITC_s recorded over the 30 trials of the shuttle-box test were expressed as mean ± SEM.

Escape failure. In the first series of experiments the dose effect of each antidepressant was evaluated by two-factor analyses of variance (ANOVAs), the factors being "doses"

TABLE 1

EFFECT OF DESIPRAMINE (A), IMIPRAMINE (B), AND AMINEPTINE (C) ON THE ESCAPE BEHAVIOR DURING THE THREE SHUTTLE-BOX SESSIONS

	Doses (mg/kg/day, IP)	*n*	Mean Number of Escape Failures		
			First Session	Second Session	Third Session
A	No exposure to inescapable shocks				
	Controls	13	10.46 ± 1.47	8.92 ± 2.32	7.54 ± 2.16
	Exposure to inescapable shocks				
	Controls	12	18.33 ± 2.50*	18.00 ± 2.39*	18 .58 ± 2.30*
	Desipramine				
	8	13	14.85 ± 1.50	18.23 ± 1.93	20.85 ± 2.70
	16	10	11.36 ± 3.50†	11.20 ± 2.98	10.90 ± 2.88†
	32	14	11.36 ± 1.67†	8.50 ± 2.03†	6.43 ± 1.46†
B	No exposure to inescapable shocks				
	Controls	12	9.08 ± 1.70	6.58 ± 2.29	6.00 ± 2.08
	Exposure to inescapable shocks				
	Controls	12	21.17 ± 1.73*	21.50 ± 2.23*	21.58 ± 1.85*
	Imipramine				
	8	10	20.50 ± 2.84	22.10 ± 2.44	21.10 ± 2.73
	16	12	10.33 ± 2.44†	10.83 ± 2.20†	11.67 ± 2.54†
	32	13	9.85 ± 1.82†	8.59 ± 1.80†	6.92 ± 2.08†
C	No exposure to inescapable shocks				
	Controls	10	9.10 ± 1.92	8.70 ± 2.39	5.40 ± 1.77
	Exposure to inescapable shocks				
	Controls	12	19.00 ± 2.88*	21.00 ± 2.53*	20.50 ± 2.61*
	Amineptine				
	8	11	18.46 ± 2.37	16.82 ± 2.40	18.64 ± 2.71
	16	12	10.25 ± 3.26†	10.42 ± 3.53†	9.67 ± 3.09†
	32	12	11.00 ± 2.84†	10.50 ± 3.11†	10.83 ± 3.01†

The drugs were administered intraperitoneally twice a day for 5 consecutive days, the first injection taking place 4 h after the inescapable shocks on day 1. Data are the mean (±SEM) escape failure number over the 30 trials of the shuttle box test.

*Indicates a significant difference between the control rats exposed to inescapable shocks and the control rats not exposed to inescapable shocks.

†Indicates a significant difference between the treated group and the controls group exposed to inescapable shocks ($p < 0.05$; two-factor ANOVAs followed by PLSD Fisher's tests).

TABLE 2

EFFECT OF AMINEPTINE ON THE NUMBER OF INTERTRIAL CROSSINGS (ITC_S) DURING THE THREE SHUTTLE-BOX SESSIONS

Doses (mg/kg/day, IP)	n	Mean Number of Intertrial Crossings		
		First Session	Second Session	Third Session
No exposure to inescapable shocks				
Controls	10	2.8 ± 0.77	2.8 ± 0.72	3 ± 0.83
Exposure to inescapable shocks				
Controls	12	1.67 ± 0.57	0.75 ± 0.25	0.58 ± 0.26
Amineptine (mg/kg/day, IP)				
8	11	1.36 ± 0.62	1.09 ± 0.72	1.36 ± 0.51
16	12	8.92 ± 2.31*†	4.58 ± 1.67†	2.50 ± 0.89
32	12	8.83 ± 2.68*†	3.82 ± 1.44	4.50 ± 2.36†

Amineptine was administered as described in Table 1.

*$p < 0.05$ between the treated group and the controls group not exposed to inescapable shocks.

†$p < 0.05$ between the treated group and the controls group exposed to inescapable shocks (Kruskall–Wallis' tests).

(between subject factor; five levels) and "daily shuttle-box session" (within subject factor; three levels). Post hoc analyses were carried out with the PLSD-Fischer's test. For the interaction studies, we were interested in assessing the influence of haloperidol or naloxone on the effects of each antidepressant, but not in comparing the effect of one antidepressant drug with the others. Accordingly, statistical analyses were performed individually for each antidepressant drug by a one-factor ANOVA, followed by the PLSD-Fischer's test for the pairwise comparisons. In the interaction studies, statistical analyses were performed on the last shuttle-box session only (day 5).

Intertrial crossings. As a previous work (19) showed an increased motor activity during intertrial intervals with dopamine stimulant drugs, we compared the dose effect of AMN with DESI and IMI on the number of ITC_s during the three shuttle-box sessions. Because these data did not display a normal distribution, the results concerning ITC were analyzed by a nonparametric test, the Kruskal–Wallis ANOVA on ranks, followed by Dunnett's test for multiple pairwise comparisons.

In all cases, the 0.05 level of significance was chosen.

RESULTS

Antidepressant Treatments (Tables 1 and 2)

Two-factor ANOVAs carried out on escape performance exhibited by rats treated with the three antidepressants tested revealed a significant "dose" effect, $F(4, 116) = 6.754$, $F(4, 108) = 11.826$, $F(4, 104) = 4.161$, for DESI, IMI, and AMN, respectively, but no "daily session" effect, $F(2, 116) = 0.875$, $F(2, 108) = 0.679$, $F(2, 104) = 0{,}784$, for DESI, IMI, and AMN, respectively. As revealed by post hoc analyses, exposure to IS significantly increased the number of escape failures by itself during the three SB sessions (Table 1). IS-induced escape deficit was reversed by the three antidepressants tested in the same range of doses (16 and 32 mg/kg/day, $p < 0.05$) (Table 1). This effect of antidepressant drugs was detected at the first SB session.

The nonparametric analyses carried out on intertrial activity revealed that DESI and IMI did not change this parameter in stressed rats whatever the shuttle-box session (data not shown). Kruskall–Wallis analyses performed in rats treated with AMN revealed a significant "dose" effect on the first shuttle-box session only ($H = 12.238$, $p < 0.05$). More precisely, the Dunnett's pairwise comparisons revealed that the number of ITC_s was significantly higher in rats treated with 16 and 32 mg/kg/day of AMN compared with stressed control and nonstressed control rats (Table 1). The effects of AMN on ITC_S gradually decreased approaching the level in the nonstressed control group, but nonetheless remained elevated relative to untreated stressed animals (Table 2).

Involvement of a Dopaminergic and/or an Opioid Mediation in the Effects of Antidepressant Treatments (Figs. 1 and 2)

Consistent with previous results, the present statistical analyses showed a significant alteration of escape behavior in stressed rats. This deleterious effect of IS was reversed by the three antidepressants used (32 mg/kg/day of AMN, DESI, IMI vs. S-C: $p < 0.01$). Haloperidol suppressed the effects of DESI and AMI assessed in the last shuttle-box test (antidepressant vs. antidepressant-haloperidol: $p < 0.01$) but not that of AMN (Fig. 1). Hal-induced reversal of DESI and IMI effects were more marked over the successive daily shuttle-box tests. DESI vs. DESI-Hal: 12.64 ± 2.60 vs. 19.31 ± 2.06 (day 3) ($p < 0.05$); 9.55 ± 2.00 vs. 16.39 ± 2.42 (day 4) ($p < 0.05$); 9.00 ± 2.43 vs. 18.92 ± 2.24 (day 5) ($p < 0.01$). IMI vs. IMI-Hal: 9.17 ± 1.63 vs. 14.63 ± 2.41 (day 3) ($p < 0.05$); 6.58 ± 1.50 vs. 14.31 ± 1.95 (day 4) ($p < 0.05$); 6.92 ± 2.10 vs. 16.13 ± 1.92 (day 5) ($p < 0.01$).

Naloxone suppressed the effect of IMI but not those of DESI and AMN assessed on the last shuttle-box test (Fig. 2). Nonetheless, Nal-induced reversal of IMI effect in stressed rats was not statistically significant in the first two shuttle-box tests. IMI vs. IMI-Nal: 7.00 ± 1.16 vs. 12.21 ± 2.56 (day 3) ($p < 0.05$); 8.33 ± 2.17 vs. 14.67 ± 3.08 (day 4) ($p < 0.05$); 5.83 ± 2.16 vs. 14.42 ± 3.09 (day 5) ($p < 0.05$).

DISCUSSION

First, these results show that AMN, a tricyclic antidepressant with DA reuptake blockade properties (7), reversed the escape deficit observed in the learned helplessness model in

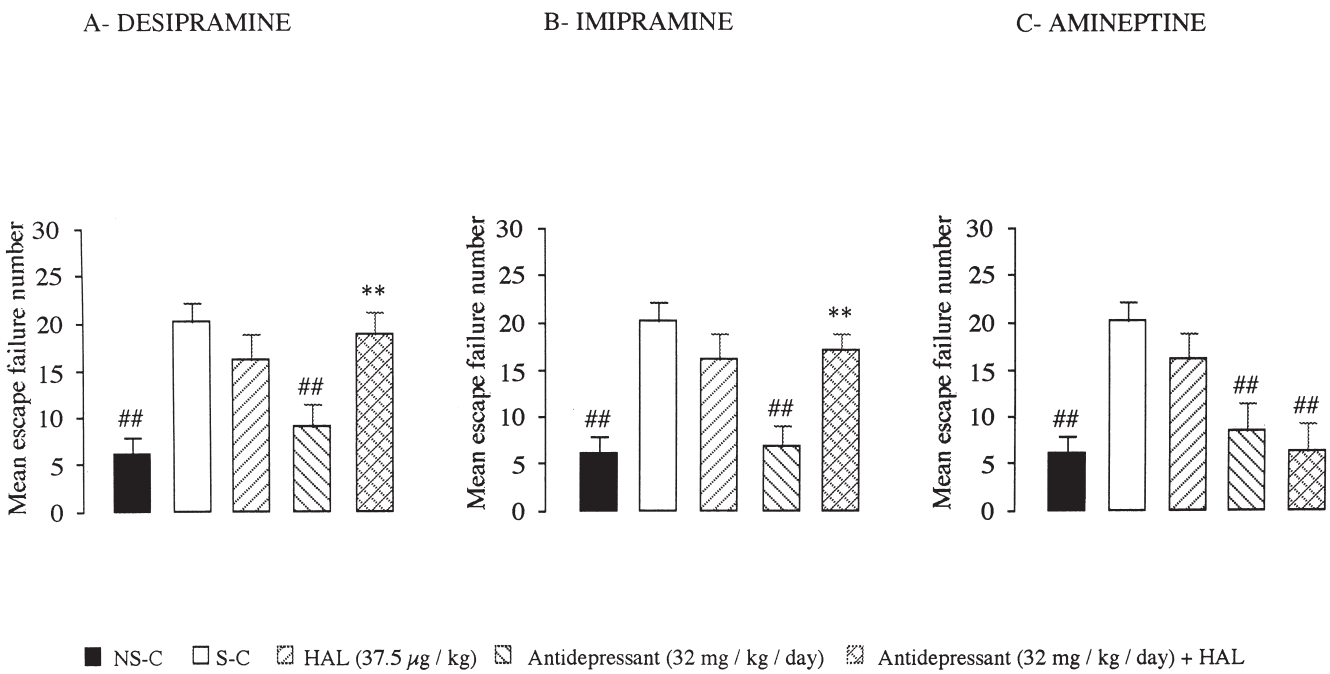

FIG. 1. Effect of an acute dose of haloperidol (37.5 μg/kg, IP) on the escape performance in the shuttle box test (day 5) of stressed rats treated with 32 mg/kg/day of an antidepressant. Stressed rats were rats exposed to inescapable shocks on day 1. Data are the mean (±SEM) escape failure number over the 30 trials of the shuttle box test. NS-C = control rats not exposed to inescapable shocks, S-C = control rats exposed to inescapable shocks, HAL = haloperidol. # indicates a significant difference from the S-C group (#$p < 0.05$, ##$p < 0.01$), * indicates a significant difference between the antidepressant-treated rats given an acute injection of haloperidol and the group treated with the antidepressant alone (*$p < 0.05$, **$p < 0.01$) (one-factor ANOVAs).

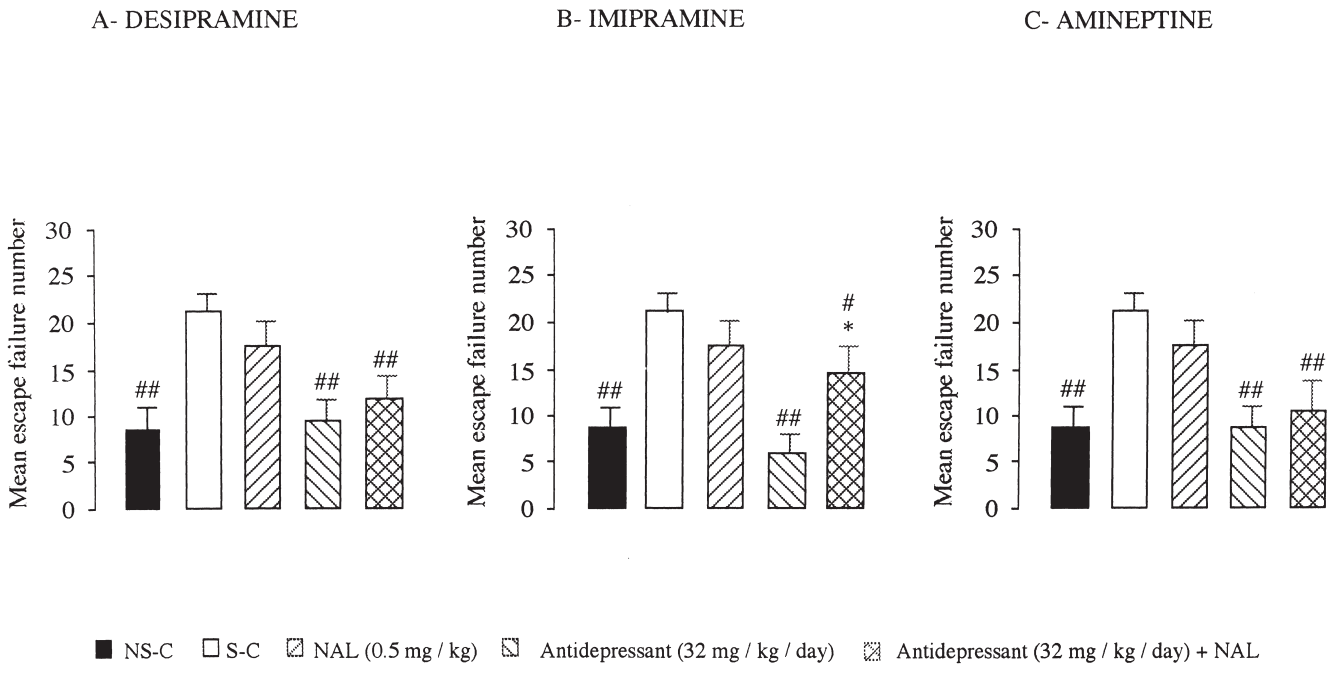

FIG. 2. Effect of an acute dose of naloxone (0.5 mg/kg, IP) on the escape performance in the shuttle-box test (day 5) of stressed rats treated with 32 mg/kg/day of an antidepressant. Stressed rats were rats exposed to inescapable shocks on day 1. Data are the mean (±SEM) escape failure number over the 30 trials of the shuttle box test. NS-C = control rats not exposed to inescapable shocks, S-C = control rats exposed to inescapable shocks, NAL = naloxone. # indicates a significant difference from the S-C group (#$p < 0.05$, ##$p < 0.01$), * indicates a significant difference between the antidepressant-treated rats given an acute injection of naloxone and the group treated with the antidepressant alone (*$p < 0.05$, **$p < 0.01$) (one-factor ANOVAs).

the same range of doses and following the same time course as IMI and DESI, two classical tricyclic antidepressants endowed with NA and mixed 5-HT/NA reuptake blockade properties, respectively (53,54). IMI and DESI (16 and 32 mg/kg/day) reversed the escape deficit without altering intertrial activity, confirming a previous report by Geoffroy and Christensen (20) with tricyclic antidepressants and MAOIs. In rats treated with 16 or 32 mg/kg/day of AMN a significant increase in ITC_s was observed during the first shuttle-box session compared with control nonstressed rats and no-drug stressed rats (Table 2). Thereafter, the intertrial activity of AMN-treated rats decreased to become statistically nondifferent from the score for control nonstressed rats, although still elevated compared with untreated stressed rats. A concomitant increase in escape performance and in ITC_s was previously reported for DA psychostimulant drugs, but no repeated shuttle-box test was used (20), and for indirect and direct opioid receptors agonists (enkephalinase inhibitors, Leu- and Met-enkephaline, morphine) for which the stimulant effects on the two parameters were maintained during the three shuttle box sessions (3,66,67). Our previous work (3) showed that in rats treated with an active dose of morphine, ITC_s, and EF_s were negatively correlated and were both affected by haloperidol, suggesting that the effects of morphine in the learned helplessness procedure were related to a psychostimulant-like activity produced by an increased DA transmission. In the present work, the number of escape responses in rats treated with AMN was stable for the same dose during the three shuttle-box tests, while the number of ITC_S decreased. Thus, an enhancement of locomotor activity cannot directly account for the improvement of escape performance produced by AMN, and the profile of action of AMN in the learned helplessness model appears to be related more to that of other tricyclic antidepressants than to that of amphetamine and opiates. This suggests that AMN, but not DESI and IMI, possesses a primary stimulant effect upon psychomotor retardation that may play a role in its antidepressant activity; the psychomotor stimulant effect disappears, whereas the antidepressant effect is maintained. This specific profile of AMN may explain its short time of action in depressed patients (7 days) compared with other tricyclic antidepressants (21 days) and its preferential efficacy in depression with anergy and inhibition (33).

Second, an acute dose of haloperidol though ineffective by itself, administered 15 min before the shuttle-box test, prevented the expression of the antidepressant-like effects of DESI and IMI. Haloperidol binds to the D_2 DA receptor and the sigma-1 receptor with similar nanomolar affinities (35,55,62). Both types of receptors are, therefore, implicated in the behavioral interaction between DESI or IMI and haloperidol in the learned helplessness procedure. However, in the forced swimming test (11,52), haloperidol antagonized the antiimmobility produced by DESI, as did sulpiride, a specific D_2 DA receptor antagonist, and fluphenazine, a neuroleptic compound with no affinity for non DA binding sites (25,30,48). Hence, it is very likely that the interaction between the two antidepressants and haloperidol in the present learned helplessness procedure occurred through D_2 DA receptors. The present results using the learned helplessness paradigm thus support those obtained with the forced swimming test, indicating that a DA mechanism is involved in the expression of the antidepressant-like effect of DESI and IMI. Because DESI and IMI have no affinity for dopamine receptor sites or for DA-uptake sites (21,53,63), the reversal of their behavioral effect by haloperidol cannot readily be explained by a direct interaction at the receptor level, but may rather be an indirect increased responsiveness of postsynaptic D_2 DA receptors. This interpretation is suggested by behavioral and microdialysis studies showing that repeated administration of DESI and IMI enhanced the response to DA receptor agonists administered systemically or directly into the terminal regions of DA neurons (39,50,59,61), and by themselves enhanced DA levels in postsynaptic regions (27,65). In addition, antidepressants have been found to increase DA release in the accumbens and the prefrontal cortex (17). Also, repeated treatment with amitriptyline and imipramine increased the affinity of D_2 DA receptors for a specific agonist in the striatum and the limbic forebrain, and increased the density of D_2 DA receptors in the latter, suggesting that repeated administration of these tricyclic compounds induced a functional D_2 DA receptor upregulation (40). However, it is possible that D_2 DA receptors are not solely involved, as recent works has shown that antidepressant drugs, IMI in particular, enhanced the responsiveness of D_3 DA receptors, probably via an increase in the density of these receptors (41).

In contrast to the above results with DESI and IMI, haloperidol did not alter the antidepressant-like effects of AMN in our experimental procedure. Consequently, the activity of AMN in the learned helplessness paradigm appears not to be mediated by a supersensitivity of D_2 DA receptors. Given the results of biochemical and binding studies indicating that AMN enhanced DA release and inhibited DA uptake (1,10), leading to higher extracellular levels of DA in postsynaptic areas (28), it is very likely that an increased DA output plays a role in its behavioral effects in the learned helplessness procedure but rather via a presynaptic mechanism and without increasing the responsiveness of D_2 DA receptors.

The interactions of antidepressant drugs with a subeffective dose (0.5 mg/kg) of naloxone were equivocal, depending on the antidepressant drug tested. The low dose of the opiate antagonist used in the present interaction study suggests that it was relatively selective for the mu-receptor subtype. An acute dose of naloxone did not change the behavioral activity of DESI and AMN, whereas it significantly antagonized the activity of IMI. The inability of naloxone to reverse the behavioral effect of DESI in our experimental procedure is at variance with the reversal by naloxone of DESI-induced antiimmobility in the forced swimming test in mice (15). This discrepancy may be explained by the different dose of naloxone (2 mg/kg vs. 0.5 mg/kg). The dose of 2 mg/kg of naloxone was not tested in the present study because we previously observed an intrinsic activity of naloxone at this dose in the learned helplessness paradigm in rats (3). The different protocol of administration of DESI (acute vs. chronic) between the two studies may also influence the results. We can also suggest that the behavioral effects of antidepressant drugs recorded in the two experimental procedures are not identically sensitive to the same neurochemical alterations. However, the lack of interaction between nomifensine, a compound with DA-uptake blockade properties, and naloxone in the forced swimming test (15) was consistent with the present results concerning the lack of interaction between AMN, another DA-uptake blocker, and naloxone. The results of Martin et al. (42), using the same experimental procedure as us, indicating a suppression by naloxone of imipramine-induced reversal of escape deficit in rats agree with our observation concerning the interaction of imipramine with opioid ligands. Unfortunately, these authors did not report the interaction of naloxone with other antidepressant drugs. A possible explanation for the difference observed between IMI and DESI may lie in the higher potency of IMI to block 5-HT uptake compared

with DESI (53), because there is evidence of interrelationships between the serotonin system and the mu-opioid system (71). The present interaction of IMI with naloxone cannot readily be accounted for by a direct interaction at opioid receptor levels because several binding studies have reported a low affinity of tricyclic antidepressants for these sites (IC_{50} = 10^{-5} to 10^{-6} M) (2,5,29,58). In the light of previous results (18), it is more likely brought about by increased levels of endogenous Met-enkephalin in the mesolimbic system. Such an effect of IMI on opioid neurotransmission might participate in its effect on the DA system described above because it is well established that mu-opioid neurons interact with DA neurons (14,32,34), and that opioid compounds enhance central dopaminergic activity (16,22,36). In any case, these findings suggest that an enhancement of mu-opioid transmission is not the main action of antidepressant treatments.

In conclusion, the present results show that daily administration of AMN, like other antidepressant drugs, counteracts the escape deficit induced by a session of inescapable shocks. They suggest that an increased DA output plays a role in this antidepressant effect of AMN, DESI, and IMI, but via a presynaptic mechanism for AMN and a postsynaptic mechanism for DESI and IMI, whereas an opioid mechanism is not a common action of these drugs, and does not seem to be necessary in the expression of their activity.

REFERENCES

1. Algeri, S.; Brunello, N.; Catto, E.; Mennini, R.; Ponzio, F.: Biochemical effect of some new proposed antidepressant drugs on the monoaminergic systems in the rat brain. In: Garattini, S., ed. Depressive disorders. Stuttgart: Schattauer Verlag; 1978:155–168.
2. Baraldi, M.; Poggioli, R.; Santi, M.; Veroni, A. V.; Bertolini, A.: Antidepressants and opiates interactions: Pharmacological and biochemical evidences. Pharmacol. Res. Commun. 15:843–857; 1983.
3. Besson, A.; Privat, A. M.; Eschalier, A.; Fialip, J.: Effects of morphine, naloxone and their interaction in the learned helplessness paradigm in rats. Psychopharmacology (Berlin) 123:71–78; 1996.
4. Besson, A.; Privat, A.M.; Eschalier, A.; Fialip, J.: Reversal of learned helplessness by morphine in rats: Involvement of a dopamine mediation. Pharmacol. Biochem. Behav. 60:519–525; 1998.
5. Biegon, A.; Samuel, D.: Interaction of tricyclic antidepressants with opiate receptors. Biochem. Pharmacol. 20:460–464; 1980.
6. Blier, P.; De Montigny, C.; Chaput, Y.: Modifications of the serotonergic system by antidepressant treatments: Implication for the therapeutic response in major depression. J. Clin. Psychopharmacol. 7:24S–35S; 1987.
7. Bonnet, J.; Chagraoui, A.; Protais, J.; Costentin, J.: Interaction of amineptine with the neuronal dopamine uptake system: Neurochemical in vitro and in vivo studies. J. Neural Transm. 69:211–220; 1987.
8. Briley, M.; Prost, J.F.; Moret, C.: Preclinical pharmacology of minalcipram. Int. Clin. Psychopharmacol. 4:9–14; 1996.
9. Carboni, E.; Tanda, G.; Frau, R.; Di Chiara, G.: Blockade of the noradrenaline carrier increases extracellular dopamine concentrations in the prefrontal cortex: Evidence that dopamine is taken up in vivo by noradrenergic terminals. J. Neurochem. 55:1067–1070; 1990.
10. Ceci, A.; Garattini, S.; Gobbi, M.; Mennini, T.: Effect of long-term amineptine on pre-and postsynaptic mechanisms in rat brain. Br. J. Pharmacol. 88:269–275; 1986.
11. Cervo, L.; Grignaschi, G.; Samanin, R.: The role of the mesolimbic dopaminergic system in the desipramine effect in the forced swimming test. Eur. J. Pharmacol. 178:129–133; 1990.
12. Chaput, Y.; De Montigny, C.; Blier, P.: Presynaptic and postsynaptic modifications of the serotonin system by long-term administration of antidepressant treatments. An in vivo electrophysiologic study in the rat. Neuropsychopharmacology 5:219–229; 1991.
13. Collu, M.; Poggiu, A. S.; Devoto, P.; Serra, G.: Behavioural sensitization of mesolimbic dopamine D_2 receptors in chronic fluoxetine-treated rats. Eur. J. Pharmacol. 322:123–127; 1997.
14. Cooper, S. J.: Interactions between endogenous opioids and dopamine: Implications for reward and aversion. In: Willner, P.; Scheel-Kruger, J., eds. The mesolimbic dopamine system: From motivation to action. Chichester: Wiley; 1991:331–336.
15. Devoize, J. L.; Rigal, R.; Eschalier, A.; Trolese, J. F.; Renoux, M.: Influence of naloxone on antidepressant drug effects in the forced swimming test in mice. Psychopharmacology (Berlin)84:71–75; 1984.
16. Di Chiara, G.; North, R. A.: Neurobiology of opiate abuse. Trends Pharmacol Sci. 13:162–185; 1992.
17. Di Chiara, G.; Tenda, G.: Blunting of reactivity of dopamine transmission to palatable food: A biochemical marker of anhedonia in the CMS model? Psychopharmacology (Berlin) 134:351–353; 1997.
18. Dziedzicka-Wasylewska, M., Rogoz, R.: The effect of prolonged treatment with imipramine and electroconvulsive shock on the levels of endogenous enkephalins in the nucleus accumbens and the ventral tegmentum of the rat. J. Neural Transm [Gen Sect]. 102:221–228; 1995.
19. Frazer, A.: Pharmacology of antidepressants. J. Clin. Psychopharmacol. 17(Suppl. 1):2S–18S; 1997.
20. Geoffroy, M.; Christensen, A. V.: Psychomotor stimulants versus antidepressants in the learned helplessness model of depression. Drug Dev. Res. 29:48–55; 1993.
21. Goodwin, G. M.: How do antidepressants affect serotonin receptors? The role of serotonin receptors in the therapeutic and side effect profile of the SSRIs. J. Clin. Psychiatry 57(Suppl. 4):9–13; 1996.
22. Gysling, K.; Wang, R. Y.: Morphine-induced activation of A10 dopamine neurons in the rat. Brain Res. 277:119–127; 1983.
23. Hamon, M.; Gozlan, H.; Bourgoin, S.; Beneliel, J. J.; Mauborgne, A.: Opioid receptors and neuropeptides in the CNS in rats treated chronically with amoxapine or amitriptyline. Neuropharmacology 26:531–539; 1987.
24. Harris, J. C.: Experimental animal modeling of depression and anxiety. Psychiatr. Clin. N. Am. 12:815–835; 1989.
25. Heroux, A. J.; Tam, S. W.; De Souza, E. B.: Autoradiographic identification of characterization of sigma receptors in guinea-pig brain using 1(cyclopropylmethyl)-4(2′(4″-fluorophenyl)-2′oxoethyl) piperidine, [^{3}H]DuP734, a novel sigma ligand. Brain Res. 598:76–86; 1992.
26. Hughes, Z.A.; Stanford, S. C.: Increased noradrenaline efflux induced by local infusion of fluoxetine in rat frontal cortex. Eur. J. Pharmacol. 317:83–90; 1996.
27. Ichikawa, J.; Meltzer, H. Y.: Effect of antidepressants on striatal and accumbens extracellular dopamine levels. Eur. J. Pharmacol. 281:255–261; 1995.
28. Invernizzi, R.; Pozzi, L.; Vallebuona, F.; Bonini, I.; Sacchetti, G.; Samanin, R.: Effect of amineptine on regional extracellular concentrations of dopamine and noadrenaline in the rat brain. J. Pharmacol. Exp. Ther. 262:769–774; 1992.
29. Isenberg, E.; Cicero, T. J.: Possible involvement of opiate receptors in the pharmacological profiles of antidepressant compounds. Eur. J. Pharmacol. 103:57–63; 1984.
30. Jenner, P.; Marsden, C. D.: Substituted benzamide drugs as selective neuroleptic agents. Neuropharmacology 20:1285–1292; 1981.
31. Jimenez-Rivera, C. A.; Segarra, O.; Santacana, G.; Hoffman, T.; Savage, D. D.; Weiss, G. K.: Chronic imipramine treatment induces downregulation of alpha-2 receptors in rat's locus coeruleus and A2 region of the tractus solitarius. Life Sci. 58:287–294; 1996.

32. Johnson, R. P.; Sar, M.; Stumpf, W. E.: A topographic localization of enkephalin on the dopamine neurons of the rat substantia nigra and ventral tegmental area demonstrated by combined histofluorescence-immunocytochemistry. Brain Res. 194:566–577; 1980.
33. Kemali, D.: Etude multicentrique de l'amineptine (Survector 100) en Italie. Clin. Neuropharmacol. 12:S44–S53; 1991.
34. Kuschinsky, K.: Psychic dependence on opioids: Mediation by dopaminergic mechanisms in the striatum? Trends Pharmacol. Sci. 4:287–289; 1981.
35. Leyssen, J. E.; Janssen, P. M.; Gommeren, W.; Wynants, J.; Pauwels, P. J.; Janssen, P. A.: In vitro and in vivo receptor binding and effects on monoamine turnover in rat brain regions of the novel antipsychotics risperidone and ocaperidone. Mol. Pharmacol. 41:494–508; 1992.
36. Magnus-Ellenbroek, B.; Havemann-Reinecke, U.: Morphine-induced hyperactivity in rat. A rebound effect. Naunyn Schmiedbergs Arch. Pharmacol. 347:635–642; 1993.
37. Maier, E.; Seligman, M. E. P.: Learned helplessness: Theory and evidence. J. Exp. Psychol. Gen. 1:3–46; 1976.
38. Maier, E.: Learned helplessness and animal models of depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 8:435–446; 1984.
39. Maj, J.; Wedzony, K.: Repeated treatment with imipramine or amitriptyline increases the locomotor response of rats to (+)-amphetamine given into the nucleus accumbens. J. Pharm. Pharmacol. 37:362–364; 1985.
40. Maj, J.; Dziedzicka-Wasylewska, M.; Rogoz, R.; Skuza, G.: Antidepressant drugs given repeatedly change the binding of the dopamine D_2 receptor agonist, [^{3}H]N-0437, to dopamine D_2 receptors in the rat brain. Eur. J. Pharmacol. 304:49–54; 1996.
41. Maj, J.; Dziedzicka-Wasylewska, M.; Rogoz, R.; Rogoz, Z.: Effect of antidepressant drugs administered repeatedly on the dopamine D3 receptors in the rat brain. Eur. J. Pharmacol. 351:31–37; 1998.
42. Martin, P.; Soubrié, P.; Simon, P.: Noradrenergic and opioid mediation of tricyclic-induced reversal of escape deficits caused by inescapable shock pretreatment in rats. Psychopharmacology (Berlin) 90:90–94; 1986.
43. Martin, P.; Soubrié, P.; Simon, P.: The effect of monoamine oxydase inhibitors compared with classical tricyclic antidepressants on the learned helplessness paradigm. Prog. Neuropsychopharmacol. Biol. Psychiatry 11:1–7; 1987.
44. Martin, P.; Thiebot, M. H.; Puech, A. J.: Modèles animaux sensibles aux antidépresseurs: Implication des systèmes sérotoninergiques et noradrénergiques centraux. Psychiatr. Psychobiol. 5:209–217; 1990.
45. Martin, P.; Guillou, N.; Lacroix, P.; Billardon, M.: Effects of the coadministration of anxiolitic and antidepressant drugs in the learned helplessness paradigm: Impact of hydroxizine. Encephale 22:270–279; 1996.
46. Muscat, R.; Papp, M.; Willner, P.: Antidepressant-like effects of dopamine agonists in an animal model of depression. Biol. Psychiatry 31:937–946; 1992.
47. Nomikos, G. G.; Damsma, G.; Wenkstern, D.; Fibiger, H. C.: Chronic desipramine enhances amphetamine-induced increase in interstitial concentrations of dopamine in the nucleus accumbens. Eur. J. Pharmacol. 195:63–71; 1991.
48. Okuyama, S.; Chaki, S.; Yae, T.; Nakazato, A.; Muramatsu, M.: Autoradiographic characterization of binding sites for [^{3}H]NE-100 in guinea-pig brain. Life Sci. 57:333–337; 1995.
49. Overmier, J. B.; Seligman, M. E. P.: Effects of inescapable shock upon subsequent escape and avoidance learning. J. Comp. Physiol. Psychol. 63:23–39; 1967.
50. Plaznik, A.; Kostowski, W.: The effects of antidepressants and electroconvulsive shocks on the functioning of the mesolimbic dopaminergic system: A behavioral study. Eur. J. Pharmacol. 135:389–396; 1987.
51. Potter, W. Z.: Adrenoreceptors and serotonin receptor function: Relevance to antidepressant mechanism of action. J. Clin. Psychiatry 57(Suppl. 4):4–8; 1996.
52. Pulvirenti, L.; Samanin, R.: Antagonism by dopamine, but not noradrenaline receptor blockers of the anti-immobility activity of desipramine after different treatment schedules in the rat. Pharmacol. Res. Commun. 18:73–80; 1986.
53. Richelson, E.: Synaptic effects of antidepressants. J. Clin. Psychopharmacol. 16:1S–8S; 1996.
54. Richelson, E.; Pfenning, M.: Blockade by antidepressants and related compounds of biogenic amine uptake into rat brain synaptosomes: Most antidepressants selectively block norepinephrine uptake. Eur. J. Pharmacol. 16(Suppl. 2):1S–7S; 1996.
55. Schotte, A.; Janssen, P. F.; Gommeren, W.; Luyten, W. H.; Van Gompel, P.; Lesage, A. S.; De Loore, K.; Leysen, J. E.: Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. Psychopharmacology (Berlin) 124:57–73; 1996.
56. Sherman, D.; Petty, F.: Neurochemical basis of the action of antidepressants on learned helplessness. Behav. Neurol. Biol. 30:119–134; 1980.
57. Sherman, D.; Sacquitne, J. L.; Petty, F.: Specificity of the learned helplessness model of depression. Pharmacol. Biochem. Behav. 16:449–454; 1982.
58. Somoza, E.; Galindo, A.; Bazan, E.; Guillamon, A.; Valenga, A.; Fuentes, J. A.: Antidepressants inhibit enkephalin binding to synaptosome-enriched fraction of rat brain. Neuropsychobiology 7:297–301; 1981.
59. Spyraki D.; Fibiger, H. C.: Sensitivity of postsynaptic dopamine receptors in the mesolimbic system after chronic administration of desipramine. Eur. J. Pharmacol. 74:195–203; 1981.
60. Stengaard-Pedersen, K.; Schou, M.: Opioid receptors in the brain of the rat following chronic treatment with desipramine and electroconvulsive shock. Neuropharmacology 25:1365–1371; 1986.
61. Stewart, J.; Rajabi, H.: Initial increases in extracellular dopamine in the ventral tegmental area provide a mechanism for the development of desipramine-induced sensitization within the midbrain dopamine system. Synapse 23:258–264; 1996.
62. Su, T. P.; Schell, S. E.; Ford-Rice, F. Y.; London, E. D.: Correlation of inhibitory potencies of putative antagonists for sigma receptors in brain. Eur. J. Pharmacol. 148:467–470; 1988.
63. Suhara, T.; Inoue, O.; Kobayasi, K.: Effect of desipramine on dopamine receptor binding in vivo. Life Sci. 47:2119–2126; 1990.
64. Tanda, G.; Frau, R.; Di Chiara, G.: Local $5HT_3$ receptors mediated fluoxetine but not desipramine-induced increase of extracellular dopamine in the prefrontal cortex. Psychopharmacology (Berlin) 119:15–19; 1995.
65. Tanda, G.; Frau, R.; Di Chiara, G.: Chronic desipramine and fluoxetine differentially affect extracellular dopamine in the rat prefrontal cortex. Psychopharmacology (Berlin) 127:83–87; 1996.
66. Tejedor-Real, P.; Mico, J. A.; Maldonado, R.; Roques, B. P.; Gibert-Rahola, J.: Effect of mixed (RB38A) and selective (RB38B) inhibitors of enkephalin degrading enzymes on a model of depression in the rat. Biol. Psychiatry 34:100–107; 1993.
67. Tejedor-Real, P.; Mico, J. A.; Maldonado, R.; Roques, B. P.; Gibert-Rahola, J.: Implication of endogenous opioid system in the learned helplessness model of depression. Pharmacol. Biochem. Behav. 52:145–152; 1995.
68. Thiebot, M. H.; Martin, P.; Puech, A. J.: Animal behavioural studies in the evaluation of antidepressant drugs. Br. J. Psychiatry 160:44–50; 1992.
69. Willner, P.: Validation criteria for animal models of human mental disorders: Learned helplessness as a paradigm case. Prog. Neuropsychopharmacol. Biol. Psychiatry 10:677–690; 1986.
70. Willner, P.: Sensitization of dopamine D2 or D3-type receptors as a final common pathway in antidepressant drug action. Clin. Neuropharmacol. 18(Suppl. 1):S49–S56; 1995.
71. Yoshioka, M.; Matsumoto, M.; Togashi, H.; Smuth, C. B.; Saito, H.: Opioid receptor regulation of 5-HT release from the rat hippocampus measured by in vivo microdialysis. Brain Res. 613:74–79; 1993.