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Comparison of Behavioral Effects of Moclobemide and Deprenyl During Forced Swimming

MARISTELA FERIGOLO, HELENA M. T. BARROS, ANDRÉ R. MARQUARDT AND MARIO TANNHAUSER

Division of Pharmacology, Federal Faculty of Medical Sciences of Porto Alegre, Sarmento Leite, 245, Porto Alegre, RS, 90050-170, Brazil

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FERIGOLO, M., H. M. T. BARROS, A. R. MARQUARDT AND M. TANNHAUSER. *Comparison of behavioral effects of moclobemide and deprenyl during forced swimming.* PHARMACOL BIOCHEM BEHAV **60**(2) 431–437, 1998.— The present study compared the antiimmobility effects of l-deprenyl (DEP) and moclobemide (MOC) to the classic antidepressant imipramine (IMI), using an ethological approach. To investigate the degree of MAO-B inhibition by DEP and MOC, combination of treatments of ineffective doses of phenylethylamine (PHEA) with DEP or with MOC were administered in three doses before immobility was tested in the forced-swimming paradigm. Tests were videotape recorded for analysis of the frequency and duration of the behaviors during the procedure. There was a significant, dose-dependent decrease in immobility duration and an increase in mobility duration of rats treated with IMI. Both active behaviors of climbing and swimming were equally enhanced by the tricyclic antidepressant, climbing behavior composing 75% of the mobile behaviors. The intermediate doses of the MAOIs tested, DEP 0.25 mg/kg and MOC 30 mg/kg, decreased immobility and increased mobility. The antiimmobility effect of DEP was due to longer climbing behavior while MOC enhanced swimming duration. No behavioral changes were seen with the administration of the lower and higher doses of the MAOI. Potentiation of the antiimmobility effects was observed when ineffective doses of PHEA and of DEP or MOC were administered in combination. Differences between the MAO inhibitors on the active behaviors were also observed when administered with PHEA; DEP and PHEA significantly increased climbing and MOC and PHEA increased swimming. This preclinical evaluation of selective MAO inhibitors indicates that both MAO-A and MAO-B inhibitors have antidepressant effects. However, to clearly demonstrate that these antiimmobility effects are a consequence of increased brain concentrations of any one of the several monoamines implicated in the mechanism of action of DEP or MOC should be the subject of future studies. © 1998 Elsevier Science Inc.

Antidepressants Monoamine oxidase inhibitors Phenylethylamine Selegiline MAO-A inhibitor MAO-B inhibitor Forced swimming

MONOAMINE oxidase inhibitors (MAOI) are classical drugs used for antidepressant treatment in clinical practice. In fact, preclinical research shows that iproniazid and nialamide decrease immobility duration of rats in the forced-swimming test, in comparison to the tricyclic antidepressants (29). However, these first-generation agents are now rarely used, due to substantial adverse effects (4).

Monoamine oxidase is found either as A or B isoenzymes, each one presenting different affinities for monoamine substrates and for which different inhibitor agents are synthesized (12). MAO-A is selectively inhibited by clorgyline and moclobemide and preferentially metabolizes norepinephrine, dopamine, octopamine, and serotonin. MAO-B metabolizes the biogenic amines phenylethylamine (PHEA), dopamine, benzylamine, and methyl-histamine, and is selectively inhibited by *l*-deprenyl, an agent also known as selegiline (10, 23,36).

Although satisfactory antidepressant activity, comparable to imipramine, and good tolerance are described in several clinical studies for moclobemide [as reviewed in (1)], some re-

Requests for reprints should be addressed to Helena M. T. Barros, Division of Pharmacology, FFFCMPA, Rua Sarmento Leite, 245, Porto Alegre, RS, Brazil.

ports deny that moclobemide is as efficacious as other antidepressants (18,32,33). In laboratory experimentation with mice, the antiimmobility effect of moclobemide in the forced-swimming test was comparable to the effects of imipramine and amitriptiline, but less than the effects of desipramine (3). On the other hand, the antidepressant effect of *l*-deprenyl is not yet well recognized in clinical practice, possibly because of the long-known belief that the antidepressant effects of MAOI is related to their action upon MAO-A and not MAO-B (12). Early studies reported deprenyl to be effective as antidepressant [for review see (11,19)]. *l*-Deprenyl showed a prompt antidepressant effect parallel to a quick and almost complete inhibition of platelet MAO-B in depressed patients (21). However, some authors could not detect any antidepressant effect when treatment with deprenyl was prescribed to mild to moderate depressive patients [e.g., (20)]. According to Mendlewicz and Youdim (21), the lack of antidepressant response seen in some studies might be explained by low MAO-B inhibition in these nonresponder patients. However, there is insufficient information about the preclinical effects of deprenyl using animal models such as behavioral despair, learned helplessness, and chronic mild stress, considered to have good predictive validity for antidepressant agents (28,35).

Inhibition of MAO-B by *l*-deprenyl increases brain concentrations of PHEA in rodents (37) and in humans (11). The combined administration of phenylalanine, a PHEA precursor, and low doses of *l*-deprenyl was effective in treatment for unipolar depression (2). Besides acting as an enzyme substrate marker of MAO-B activity, PHEA may also play a role in the clinical recovery after tricyclic antidepressant treatment (31). Although selective, moclobemide does not display a specific MAO-A inhibitor profile. Two to 4 h after the drug is given, one of its metabolites (RO 16-6491) may inhibit 40% of MAO-B activity in rodent brain (6). The antiimmobility effect of moclobemide has been attributed to the increases in concentration of norepinephrine, dopamine, or serotonin that occur within the first hour of drug administration (22). However, because better antiimmobility effects are generally seen 24 h after dosing (29), which is time enough to allow for accumulation of this active metabolite of moclobemide, more reliable experimental results are expected if the traditional dosing schedule is used, i.e., the agent is administered 24 h, 5 h, and 1 h before retest (20).

The objective of the present study was to compare the effects of multiple doses of *l*-deprenyl and moclobemide on the whole set of acts and postures of rats submitted to the forced-swimming test. To allow the description of the effects of each MAOI chosen on all active behaviors presented during the test procedures, videotaping and categorizing types of behaviors was chosen. A dose–response study for imipramine was also conducted to allow for comparisons with a prototype substance. A possible interaction between PHEA and moclobemide or *l*-deprenyl was tested using the same paradigms, to evaluate the possible participation of MAO-B in these drugs effects.

METHOD

Animals

One hundred fifty-eight male Wistar rats 80–90 days old were obtained from the colony of the Federal Faculty of Medical Sciences of Porto Alegre. The rats were housed in 50 × 36 × 18 cm plastic cages, in groups of 5, at a room temperature of 22 ± 2°C under a constant light–dark cycle (lights on from 0700–1900 h). Rodent chow (Nutrilab, Brazil) and water were available at all times. During breeding, housing and experimental procedures current national laws and the NIH Guide for Care and Use of Laboratory Animals were followed.

Drugs

Solutions of 5, 10, and 20 mg/ml of beta-phenylethylamine hydrochloride (Sigma, St. Louis, MO); 10, 30, and 60 mg/ml moclobemide (Roche, Brazil), and 2.5, 5, and 10 mg/ml imipramine hydrochloride (donated by Biogalenica, Brazil) were prepared in saline and administered intraperitoneally (IP). *l*-Deprenyl (selegiline hydrochloride; donated by Knoll, Brazil) 0.1, 0.25, and 0.5 mg/ml solutions were also prepared in saline and were administered subcutaneously (SC). Saline (sodium chloride 0.9 g/ 100 ml; Labsynth, Brazil) was used as control solution. All solutions were administered in a fixed volume of 1 ml/kg.

Forced-Swimming Test

Rats were individually forced to swim in a covered aquarium (25 × 25 × 40 cm), containing 28 cm of water at 25°C. This volume of water precluded rats touching the bottom with their feet or tails. Rats were submitted to the procedure for 15 min on the first day (pretest) and for 5 min on the second day (test), 24 h later. Each rat received the drug administrations 24 h, 5 h, and 1 h before the retest (27,29). PHEA treated groups received an additional injection 5 min before the retest. After each swimming session the rats were thoroughly dried with towels and warmed under a heat source. The pretest sessions of the control group animals and all retest sessions were videotape recorded for further analysis.

For each of the drugs tested, separate experiments were conducted. Increasing doses of PHEA (control, 5, 10, and 20 mg/kg), moclobemide (control, 10, 30, and 60 mg/kg), or deprenyl (control, 0.1, 0.25, and 0.5 mg/kg) and imipramine (control, 2.5, 5, and 10 mg/kg) were administered. Each group was composed of eight rats. A separate experiment, where animals were treated with PHEA 10 mg/kg, alone or in combination with DEP 0.1 mg/kg or MOC 10 mg/kg was also performed. These doses were chosen because they had not produced antiimmobility effects in the former experiments. Groups of 10 rats each were formed for the interaction study.

To discard false positive effects due to increased motor activity induced by stimulant effects of drugs (29), animals treated with drug doses used in the above-described experiments were observed in an open field, for 5 min. Locomotion, rearing, grooming, and defecation were estimated using a hand counter.

Behavioral Analysis

Behavioral analysis was conducted by two previously trained observers, who had similar rating performance at the 95% confidence limit for each one of the behaviors. The values used were the mean ratings given by each researcher. Videotapes were analyzed through direct computer keyboard input to a Basic written software. The key encoding the behavior observed was depressed by the observer and the duration and frequency data of immobility, diving, motility, and head shake were measured throughout the whole duration of the experiment. Diving was counted every time the rat's whole body was under water. Mobility was counted when the animals showed vigorous struggling movements while in the middle of the water or close to the borders and while trying to climb the walls. Immobility was counted each time the animal

was floating, with the face above the water surface and making only slight movements with the front paws to keep from submerging. Head shakes were defined as an abrupt horizontal movement of the head. The mobility periods were further evaluated to differentiate between climbing (when the rat was close to the wall and making active up and down movements with the forepaws forcing the body upwards) and swimming (whenever the animal was moving with a gliding motion while in the middle of the water or moving around in the container).

Statistics

The results of the mobility and immobility parameters are presented as mean ± standard error of the mean of frequency and of duration of each behavioral display during the period of observation. Data referring to diving and head shakes were analyzed considering their frequencies. Each set of experimental results was analyzed using one-way ANOVA, with the drug dose as the independent factor. Student–Newman–Keuls test was used to verify further contrasts between doses. Significant differences were considered when $p < 0.05$.

RESULTS

To validate a full-time ethological evaluation of the forced-swimming test, assessment of behaviors during the whole procedure time of pretests and retests of control animals of our experiments was initially performed. Considering the behavior of the control animals in all experiments in the 15-min pretests, 69.9 ± 2.5% of the time was spent in immobile behavior. Mean duration of immobility was 629.4 ± 22.1 s, and of mobility was 270.3 ± 22.1 s. The frequencies for the observed behaviors were 5.8 ± 0.4 for immobility and 5.8 ± 0.45 for mobility during the 15-min sessions. Diving occurred 3.21 ± 0.54 times and head shakes occurred 47.1 ± 5.3 times during the test session. The frequency of alternation between mobility and immobility behaviors gradually decreased as time lapsed, the animals tending to remain much more immobile. The frequency of head shakes and diving behavior also decreased with time.

For the analysis of average time spent on each behavior only mobility and immobility were of sufficient duration for measurement. Diving and head shakes were very rapid active behaviors, lasting less than 1 s each and, therefore, contributed very little to the overall time. The duration of mobility decreased as a consequence of increased duration of immobility. For that reason, mobility appeared as a mirror image of immobility and was considered to have lower prominence than immobility for results presentation, even though it was always analyzed separately. This behavior is very important as an internal check for accuracy of scoring.

During the 5 min of retest, the frequency and the duration of mobile and immobile behaviors were similar to the first 5 min (0–300 s) of the first day pretest. The control animals showed immobility during 41.7 ± 3.6% of the time of the 5-min retest with the counterpart mobility corresponding to 58.3 ± 3.6% of the retest time. Diving during the retest (0.32 ± 0.2) occurred significantly less often than diving on the first 300 s of the pretest (2.86 ± 0.5). However, the number of head shakes during the 5-min retest was not significantly different from the pretest day (32.8 ± 3.5).

The effects of IMI and MAOIs on the duration of immobility during retest trials are represented in Table 1. IMI increased mobility duration at all doses; however, only the doses of 2.5 and 10 mg/kg showed significant differences when compared to the respective control groups, $F(3, 28) = 5.16$, $p < 0.01$. IMI 10 mg/kg also showed a decrease of immobility time. MOC 30 mg/kg significantly increased mobility duration, $F(3, 28) = 3.84$, $p < 0.02$, and decreased immobility. The intermediate dose tested of DEP (0.25 mg/kg) also increased mobility, $F(3, 28) = 3.43$, $p < 0.05$) and decreased immobility duration.

Treatments with IMI and with MOC 30 mg/kg decreased the number of times rats engaged in immobile behavior, because they increased the time spent in mobility behavior.

TABLE 1

IMIPRAMINE (IMI), MOCLOBEMIDE (MOC), AND L-DEPRENYL (DEP) DECREASE TIME SPENT IMMOBILE

Treatment	mg/kg	Immobility Duration	Immobility Frequency	Head-Shake Frequency
CON	0	134.8 ± 16.2	4.50 ± 0.66	27.8 ± 3.46
IMI	2.5	77.8 ± 17.5*	3.63 ± 0.54	29.9 ± 4.22
IM	5	109.3 ± 20.4	2.63 ± 0.37*	21.0 ± 4.18
IMI	10	50.9 ± 13.2*	1.31 ± 0.28*	12.0 ± 1.28*
$F(3, 28) =$		**4.60**	**7.90**	**5.32**
CON	0	150.2 ± 25.1	4.75 ± 1.09	36.0 ± 5.91
DEP	0.1	186.2 ± 20.9	3.06 ± 0.58	31.0 ± 4.95
DEP	0.25	82.3 ± 19.9*	3.88 ± 0.54	24.4 ± 3.67
DEP	0.5	124.4 ± 27.9	4.13 ± 0.75	34.3 ± 3.42
$F(3, 28) =$		**3.41**	*0.83*	*1.24*
CON	0	134.8 ± 10.5	4.56 ± 0.87	34.8 ± 5.14
MOC	10	107.7 ± 21.0	3.13 ± 0.58	32.6 ± 5.27
MOC	30	51.4 ± 18.7*	1.31 ± 0.43*	21.6 ± 4.05
MOC	60	132.6 ± 21.4	2.88 ± 0.54	15.3 ± 2.44*
$F(3, 28) =$		**3.43**	**4.51**	**4.43**

IMI and MOC decrease immobility and head-shake frequencies during the forced-swimming test.

Bold F-values represent $p < 0.05$ *differs from control $p < 0.05$; groups of eight rats each.

TABLE 2

LOW DOSES OF COMBINED TREATMENT WITH PHENYLETHYLAMINE (PHEA) AND DEPRENYL (DEP) AND PHEA AND MOCLOBEMIDE (MOC) DECREASE TIME SPENT IMMOBILE AND FREQUENCIES OF IMMOBILITY AND HEAD-SHAKES DURING THE FORCED SWIMMING TEST

Treatment	mg/kg	Immobility	Immobility	Head Shakes
CON	0	144.9 ± 16.7	4.31 ± 0.93	41.8 ± 7.00
PHEA	5	181.8 ± 17.8	5.50 ± 1.05	44.5 ± 2.53
PHEA	10	174.2 ± 20.3	6.06 ± 0.43	40.7 ± 5.96
PHEA	20	89.6 ± 15.1*	6.13 ± 0.54	29.5 ± 4.95
$F(3, 28) =$		**5.56**	*0.97*	*1.52*
CON	0	104.4 ± 17.6	4.70 ± 0.81	21.55 ± 3.28
PHEA + DEP	10 + 0.1	16.4 ± 9.14*	0.70 ± 0.40*	8.25 ± 2.87*
PHEA + MOC	10 + 10	7.1 ± 3.51*	0.95 ± 0.40*	13.10 ± 3.36
$F(2, 27) =$		**18.9**	**15.2**	**4.49**

Bold *F*-values represent $p < 0.05$; *differs from control $p < 0.05$; groups of 10 rats each.

Head shakes were significantly decreased after treatment with IMI 10 mg/kg and with MOC 60 mg/kg. DEP did not induce changes in the frequency of any of the behaviors evaluated. Diving was not modified by any of the drug treatments given, remaining rare during the retest sessions.

Shorter immobility duration as seen in Table 2 and longer mobility duration, $F(3, 28) = 5.55$, $p < 0.01$, were seen after treatment of rats with the higher dose of PHEA tested (20 mg/kg). The doses of PHEA 10 mg/kg, DEP 0.1 mg/kg, and MOC 10 mg/kg for the interaction part of this study were determined by their lack of behavioral effects on the forced-swimming test. Combined treatment of PHEA 10 mg/kg and DEP 0.1 mg/kg or PHEA 10 mg/kg and MOC 10 mg/kg significantly increased mobility duration, $F(2, 27) = 18.7$, $p < 0.0001$) and decreased immobility duration when compared to the control group.

None of the doses of PHEA modified the frequency of immobility, mobility, and head shakes. However, after PHEA 10 mg/kg + DEP 0.1 mg/kg or PHEA 10 mg/kg + MOC 10 mg/kg there was a decrease of mobility frequency, $F(2, 27) = 14.1$, $p < 0.0001$, and of immobility frequency, $F(2, 27) = 15.2$, $p < 0.0001$, reflecting the much longer duration of mobility engaged in by animals almost immediately after they are put in the water.

The results in Table 3 show that the control animals were climbing during around 75% of the time spent in mobile behavior during the retest. For all the treatments with isolated dosing that decreased immobility, total time spent in active mobile behaviors was represented by more climbing behavior than swimming. The contribution of climbing and swimming to mobility after antidepressant administration kept the same proportions as for the controls. A significant increase in climbing was only seen after the administration of PHEA 10 mg/kg + DEP 0.1 mg/kg, when climbing contributed to 88% of the time spent mobile. A significant increase in swimming was seen after the combined administration of PHEA 10 mg/kg and MOC 10 mg/kg, when swimming took place for 31% of the mobility time.

None of the drug doses tested increased locomotion, rearing, grooming, or fecal bolus parameters of the animals observed in the open field. Control-treated animals presented locomotion of 41.8 ± 5.3; median of rearing of 15; median of grooming of 1.5 and 2.7 fecal boluses. MOC 30 mg/kg significantly decreased locomotion to 28.2 ± 2.8, $F(3, 28) = 5.58$; $p < 0.05$, and when MOC 10 mg/kg was given combined to PHEA 10 mg/kg a decrease in rearing behavior was also detected ($H = 18.37$, $p < 0.05$). Combined administration of PHEA 10 mg/kg and DEP 0.1 mg/kg decreased all behaviors in the open field: locomotion was decreased to 12.2 ± 3.9, $F(3, 28) = 3.92$, $p < 0.05$; rearing to 1.0 ($H = 15.03$, $p < 0.05$); and the animals did not groom or defecate during the observation. The doses used in these experiments did not produce any sign of stimulation such as stereotyped behavior that could have interfered with the exploratory activity measured in the open-field test or with the behaviors during the forced-swimming test.

DISCUSSION

The present study shows that both of the selective MAO inhibitors tested, moclobemide and *l*-deprenyl, resulted in U-shaped dose–response curves when immobility duration was considered in the forced-swimming test. Only the intermediate doses of deprenyl (0.25 mg/kg) and of moclobemide (30 mg/kg) shortened immobile behavior, in proportions comparable to the antiimmobility effect of IMI 10 mg/kg.

Tricyclic and atypical antidepressants and the nonselective MAOIs are recognized to decrease rats immobility duration in the forced-swimming procedure (8,27,29). There is a corre-

TABLE 3

DURATION (MEAN + SEM) OF CLIMBING AND SWIMMING BEHAVIORS AFTER THE ADMINISTRATION OF IMIPRAMINE (IMI) 10 mg/kg, L-DEPRENYL (DEP) 0.25 mg/kg, MOCLOBEMIDE (MOC) 30 mg/kg, PHENYLETHYLAMINE (PHEA) 20 mg/kg, PHEA 10 mg/kg + MOC 10 mg/kg, AND PHEA 10 mg/kg + DEP 0.1 mg/kg

Treatment	mg/kg	Climbing	Swimming
CON	0	124.25 ± 17.34	40.25 ± 8.38
IMI	10	145.25 ± 19.18	52.00 ± 5.58
DEP	0.25	178.50 ± 22.34	7.25 ± 2.89
MOC	10	179.87 ± 26.00	29.62 ± 9.74
PHEA	20	182.12 ± 21.01	20.50 ± 9.74
PHEA + DEP	10 + 0.1	232.00 ± 16.85*	40.70 ± 13.06
PHEA + MOC	10 + 10	187.60 ± 30.35	83.80 ± 26.95†
$F(6, 59)$		**2.314**	**2.971**

Bold *F*-values represent $p < 0.05$; *differs from control $p < 0.05$; †differs from PHEA $p < 0.05$.

lation between the antiimmobility potency of the antidepressants and their clinical efficacy justifying the predictive validity of the forced-swimming test (35). However, only recently the ethological evaluation of this procedure was introduced with the purpose of defining differences between antidepressants, using their diverse effects on different active behaviors of rats during the forced-swimming test (8). The full-time sampling analysis employed by us to evaluate behaviors during the forced-swimming adds valuable information to the procedure. It shows that the animals treated with IMI dose dependently engage less frequently in immobile behavior and, consequently, mobility episodes become longer. The evaluation of the subacute treatment with IMI confirmed the dose-dependent decrease in duration of immobility (27,29). However, differing from desipramine (8), another tricyclic antidepressant, which increased climbing, imipramine produced a mixed effect, slightly increasing both mobile behaviors, climbing and swimming, and only significantly increasing total mobility. As a 5-HT reuptake inhibitor one would expect imipramine to increase swimming, but this mixed effect is probably explained by its metabolism into desipramine, in mammals, which blocks norepinephrine reuptake and increases climbing (8). Another behavior frequently shown by rats during the forced-swimming is head shakes, considered to be a consequence of "pina reflex" (27,29). Curiously, IMI significantly decreased head shakes, in a similar proportion to the decrease in immobility. Because this behavioral effect might be helpful in the study of 5-HT_2 receptor functions (34) the underlying mechanism for this behavioral observation deserves further attention.

It was proposed that agents with predominant norepinephrine mechanisms of action would increase climbing, while the serotonergic acting antidepressants would increase swimming (8). Consequently, one would expect moclobemide to increase 5-HT transmission, especially when combined to PHEA, because it decreases immobility mainly by increasing swimming. In fact, moclobemide was recently shown to significantly increase 5-HT and 5-HIAA concentrations in cortex, cerebellum, striatum, thalamus, and brain stem and to avoid the decrease of norepinephrine induced by forced-swimming in mice (18,22). Similar findings were described for rats in which whole brain serotonin concentrations were increased after one dose of moclobemide, while norepinephrine concentrations were increased after prolonged treatment with moclobemide 10 mg/kg (5).

On the other hand, *l*-deprenyl decreased immobility mainly by intensification in climbing behavior, which leads to the expectation of an increase in norepinephrine activity in cerebral areas. Knoll and collaborators proposed that *l*-deprenyl is a catecholaminergic activity enhancer. Following in vitro incubation with very low concentrations of *l*-deprenyl increased the release of dopamine by 30 to 90% in striatum, substantia nigra, and tuberculum olfatorium and increased the release of norepinephrine to more than 100% from control levels in locus coeruleus (16). Increased serotonin release from raphe could only be detected after longer incubation session of cerebral tissue with much higher doses of deprenyl (16).

In addition to the study of the motor patterns apparently related to an active search of a way out, the use of frequency of the immobile posture and of head shakes also differentiated the two selective MAOI tested here. Moclobemide produced a similar effect as IMI. The doses of both agents that decreased immobility duration also decreased its frequency and decreased the number of head shakes. Deprenyl differed from the other compounds because its only discernible effects were on the duration of behaviors. In this respect it shows similarity to PHEA, which did not interfere with immobile posture frequency and did not modify the number of head shakes. The similar behavioral profiles of PHEA and *l*-deprenyl may be justified by the important increase in PHEA brain concentrations when *l*-deprenyl is given (37).

Decreased immobility after intraperitoneal or oral administration of moclobemide to mice in doses ranging from 3 to 100 mg/kg, has been previously described (3,17). Therefore, demonstration of an antiimmobility effect of moclobemide in rats is not surprising. However, the effective dose range in these animals seems narrower than for mice and could be dependent on species differences or the routes of administration used in the different studies. The observation that only intermediate doses of the MAOI induced an antiimmobility effect could also explain why some studies do not detect the antidepressant effect of moclobemide or of deprenyl in humans (20). In our study, the antiimmobility effect of deprenyl occurred with the 0.25 mg/kg dose, which is the highest selective dose to inhibit MAO-B in rats brains, while deprenyl 0.5 mg/kg SC starts to lose its selective effect (11,13,14). It is possible that the administration of elevated doses of deprenyl determines the presence of higher levels of active metabolites in the central nervous system (30), blunting the main behavioral effects of the drug.

Knoll and collaborators recently proposed that very low doses of deprenyl, in the range of 0.01 to 0.25 mg/kg, have a catecholamine enhancing effect, not necessarily related to MAO-B inhibition. This effect can be seen after only one low dose of the agent and no tolerance is detected up to 3 weeks of continued deprenyl dosing (15,16). This catecholaminergic enhancer effect is not secondary to a tyramine-like release effect, nor to a reuptake blocker effect, but rather seems to occur due to stimulation of action-potential transmitter release coupling in noradrenergic and dopaminergic neurons (16).

Clinical findings in favor of the proposal of mood modulation by PHEA were reviewed recently (7). Using the swim-immobility test our study depicts antidepressant drug-like effects after the systemic administration of PHEA. These results agree with those of clinical and experimental studies such as the presence of lower urine concentrations of PHEA in depressive patients than in normal individuals (9,31). On the other hand, the antiimmobility effect of PHEA could be secondary to its similarity to amphetamines (25). However, according to previous results from our laboratory the dose of PHEA with antiimmobility effect is three times lower than the dose producing increased motor behavior and stereotypies in rats, discarding a false-positive observation (27,29). PHEA was proposed to act as a neuromodulator of catecholamine transmission, increasing the release of dopamine and norepinephrine (17,24,26). If PHEA's metabolic route is decreased in depression, norepinephrine and dopamine postsynaptic effects are expected to be decreased or less arousable (26).

If there is a role for PHEA in mood modulation, the effect would be expected to increase when its metabolizing enzymes are blocked. Synergism between ineffective doses of deprenyl and moclobemide with PHEA was detected, with immobility duration decreased to less than 15% of the control values. Deprenyl is a MAO-B inhibitor by itself, selectively inhibiting PHEA deamination and increasing its tissue concentrations (12). Although moclobemide is recognized as a selective MAO-A inhibitor, it is biotransformed into metabolites that inhibit MAO-B. In rats, an active metabolite (Ro 16-6491) is detectable within 30 min of the administration of the drug and can be found for hours in circulation (32). Also, in human

platelets up to 50% of MAO-B inhibition can be detected after the administration of a single dose of moclobemide (6). Other nonselective MAOI and antidepressant agents not belonging to the MAOI group also are described to increase PHEA levels in human urine and rat brain (7,37). So, in spite of the in vitro selectivity of drugs as MAO-A subtype inhibitors some in vivo effects may induce some interaction with endogenous substances such as PHEA. The involvement of PHEA in depression is much less clear than for serotonin, norepinephrine, or even dopamine; however, PHEA is also proposed to act as a catecholaminergic enhancer, increasing the release of norepinephrine and dopamine in brain (17,26), which could explain its antidepressant effect.

It is tempting to relate the behavioral effects of certain doses of MAO-B inhibitors to the catecholamine enhancer effect of the pharmacological agent itself, to the increased brain tissue PHEA or to the combination of these two cerebral tissue effects. It would be extremely interesting and necessary to demonstrate that the antiimmobility effects of deprenyl and moclobemide occur in parallel with selective changes of any one of the monoamines (serotonin, dopamine, norepinephrine, or PHEA) levels in brain areas, to help elucidate their antidepressant mechanism of action. The clinical interest of such findings may be its utility to define the individual differences between depressive patients that are distinguished by different degrees of therapeutic response when treated with agents with selective activity upon serotonergic or catecholaminergic systems.

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