Clonidine potentiates the effects of tranylcypromine, phenelzine and two analogues in the forced swimming test in mice

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Objective: To compare tranylcypromine (TCP) and phenelzine (PLZ), two well-established inhibitors of monoamine oxidase (MAO), and 2 of their analogues, 4-fluorotranylcypromine (FTCP) and N^2-acetylphenelzine (AcPLZ) respectively, with regard to effects in the forced swimming test, a behavioural test used to screen for potential antidepressant drugs. **Methods:** Mice were dropped individually into glass cylinders containing water. The duration of their immobility was scored during the last 4 minutes of the test. **Results:** Except for TCP at high doses, none of the drugs demonstrated activity when administered alone. All 4 drugs were active when given in combination with clonidine, an effect thought to be the result of mixed action at 5-HT_{1A} and 5-HT_2 receptors and the noradrenergic system. 5-HT_{1B} receptors do not seem to be implicated, as lithium did not potentiate the effect of any of the drugs. Quinine activation of AcPLZ suggests that this analogue acts on 5-HT_3 receptors. **Conclusions:** FTCP and AcPLZ demonstrated anti-immobility activity in the forced swimming test when used in association clonidine. These findings confirm previous neurochemical findings suggesting that these drugs have antidepressant properties.

Objectif : Comparer la tranylcypromine (TCP) et la phénelzine (PLZ), deux inhibiteurs bien connus de la monoamine oxydase (MAO), et deux de leurs analogues, la 4-fluorotranylcypromine (FTCP) et la N^2-acétylphénelzine (AcPLZ) respectivement, en ce qui concerne les effets dans le cadre du test de la nage forcée, test de comportement utilisé pour dépister la présence d'antidépresseurs possibles. **Méthodes** : On a laissé tomber individuellement des souris dans des cylindres de verre remplis d'eau. On a noté la durée de leur immobilité au cours des quatre dernières minutes du test. **Résultats** : Sauf dans le cas de la TCP à fortes doses, aucun des médicaments n'a produit d'activité lorsqu'on l'a administré seul. Les quatre médicaments étaient actifs lorsqu'on les a combinés avec la clonidine, effet qui découle, croit-on, de

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l'action mixte aux récepteurs de la 5-HT_{1A} et de la 5-HT_2 et du système noradénergique. Les récepteurs de la 5-HT_{1B} ne semblent pas impliqués, car le lithium n'a accentué l'effet d'aucun des médicaments. L'activation par la quinine de l'AcPLZ indique que cet analogue agit sur les récepteurs de la 5-HT_3. **Conclusions** : La FTCP et l'AcPLZ ont montré une activité anti-immobilité dans le contexte du test de la nage forcée lorsqu'on les a associées à la clonidine. Ces résultats confirment des constatations neurochimiques antérieures qui indiquent que ces médicaments ont des caractéristiques antidépressives.

Introduction

The monoamine oxidase (MAO) inhibitors, although not used as extensively as some other antidepressants, have an important role in the treatment of atypical depression and depression associated with anxiety, agitation and phobia. Tranylcypromine (TCP) is an antidepressant drug classified as an irreversible, nonhydrazine, nonselective inhibitor of MAO (i.e., it inhibits both the A and the B forms of MAO). This multifaceted drug has other actions that may contribute to its overall profile, including effects on catecholamine and serotonin (5-HT) reuptake and release; down-regulation of β-adrenergic, 5-HT_2 and tryptamine receptors; and effects on enzymes other than MAO.[1,2] Phenelzine (PLZ) is also a potent irreversible, nonselective inhibitor of MAO. Both TCP and PLZ have been used extensively in psychiatry for many years as antidepressants and, particularly in the case of PLZ, in the treatment of several anxiety disorders, including panic disorder and social anxiety disorder. In addition to elevating brain levels of a number of biogenic amines, including the catecholamines and 5-HT, by inhibiting MAO, PLZ also inhibits γ-aminobutyric acid (GABA) transaminase and, thus, elevates brain levels of GABA.[3–5]

In an effort to improve the pharmacokinetic properties of TCP, we synthesized a number of analogues in which the para position of the phenyl ring was substituted, which prevents metabolism at this site. We found that 4-fluorotranylcypromine (FTCP) is a more potent inhibitor of MAO-A and MAO-B in the rat brain than the parent drug (TCP), both in vitro and ex vivo.[6] After injection at equimolar doses, FTCP attained more sustained brain levels than TCP.[2,7] In several neurochemical screens this analogue has shown properties characteristic of antidepressants. N^2-Acetylphenelzine (AcPLZ) has been identified unequivocally as a metabolite of PLZ in humans and rats, but on the basis of its concentrations in tissues and body fluids, it appears to be a very minor metabolite.[8-10] However, other data indicate that it is a useful pharmacological tool and may, in fact, have antidepressant properties in its own right. Like PLZ, AcPLZ inhibits MAO and elevates brain levels of biogenic amines.[5,11] However, in contrast to PLZ, it does not inhibit GABA transaminase or elevate GABA levels in the brain.[5] It is thus conceivable that AcPLZ could be an effective MAO-inhibiting antidepressant with a better side-effect profile than the parent drug. Despite the neurochemical evidence suggesting that AcPLZ has antidepressant properties, to our knowledge it has never been tested in an animal model of depression. It was thus of considerable interest to compare the parent drugs TCP and PLZ with FTCP and AcPLZ in a screening test for antidepressants.

The forced swimming test (FST) is a behavioural test used to predict the efficacy of antidepressant treatments.[12] It has good predictive value for antidepressant potency in humans.[13] In previous studies with the FST, combining clonidine with subactive doses of several antidepressants that were inactive when administered alone produced significant anti-immobility effects in mice.[14,15] Pretreatment with lithium has an additive effect on some antidepressants in this test, particularly those that act on 5-HT systems;[16] this additive effect was proposed to result from inhibition of potassium channels.

The present study was performed to evaluate the antidepressant-like activity of FTCP and AcPLZ in mice and to compare this activity with that of the parent drugs, TCP and PLZ. Clonidine, lithium and quinine were used in combination with the 4 MAO inhibitors to further elucidate their mechanism or mechanisms of action.

Method

Animals

Naive male Swiss mice (Centre d'élevage Janvier, Le Genest, France), weighing 20–24 g, were housed at constant room temperature (20–22°C) under standard conditions, with free access to food and water. Each experimental group consisted of 10 randomly chosen mice.

The ethical rules of the French Ministry of Agricul-

ture for experiments with laboratory animals (bylaw no. 87.848) were followed.

Drugs and treatments

The following drugs were used in the study: TCP hydrochloride (RBI-Sigma, Saint Quentin Fallavier, France), PLZ sulphate (RBI-Sigma), clonidine hydrochloride (RBI-Sigma), lithium gluconate (Labcatal, Montrouge, France) and quinine hydrochloride (RBI-Sigma). FTCP and AcPLZ were synthesized in the Neurochemical Research Unit, University of Alberta, Edmonton.[6,10]

The MAO inhibitors were dissolved in a 1% aqueous solution of Tween 80 (Merck, Nogent sur Marne, France), and all other drugs were dissolved in distilled water. The drugs were injected intraperitoneally, in a standard volume of 0.5 mL/20 g body weight, 45 minutes (for the interacting agents clonidine, lithium and quinine) or 30 minutes (for the MAO inhibitors) before the test. Every experiment included a vehicle control, interacting agent controls and MAO inhibitor controls.

Subactive doses of the interacting agents were based on previous studies from our laboratories.[14,16,17]

Measurement of immobility in mice

The FST used here was essentially the same as described in detail elsewhere.[18] Mice were dropped individually into glass cylinders (height 25 cm, diameter 10 cm) containing a depth of 10 cm of water maintained at 23–25°C and were left in the water for 6 minutes. A mouse was judged immobile if it floated in the water in an upright position and made only small movements to keep its head above water. The duration of immobility was recorded during the last 4 minutes of the 6-minute test by 2 trained experimenters.

Statistics

For each group of mice, the mean period of immobility and the standard error of the mean (SEM) were calculated. The effects of the MAO inhibitors combined with the interacting agents were also expressed as the percentage change from the MAO inhibitors alone.

The effects of the MAO inhibitors on mouse behaviour in the FST were determined by a 1-way analysis of variance (ANOVA) and Dunnett's test. The effects of clonidine, lithium or quinine on the action of the 4 MAO inhibitors were determined by 2-way ANOVA and Sidak's test.

Results

Effects of MAO inhibitors: dose–response studies

Administration of TCP resulted in anti-immobility effects at doses of 4 and 8 mg/kg ($p < 0.05$ and $p < 0.01$ respectively) (Table 1). FTCP did not affect the period of immobility. Mice treated with doses of TCP and FTCP higher than 8 mg/kg were unable to swim because of severe side effects.

PLZ and AcPLZ did not modify the period of immobility (Table 1).

Table 1: Effects of 4 monoamine oxidase (MAO) inhibitors on performance of mice in the forced swimming test

Dose, mg/kg	MAO inhibitor; mean period of immobility (and SEM), s, and % of zero dose											
	Tranylcypromine			4-Fluorotranylcypromine			Phenelzine			N^2-Acetylphenelzine		
0.0	231	(3)	100	233	(2)	100	222	(5)	100	232	(3)	100
0.5	204	(8)	88	228	(3)	98	ND			ND		
1.0	223	(4)	97	226	(3)	97	227	(4)	102	237	(1)	102
2.0	214	(12)	93	208	(17)	89	223	(5)	100	220	(10)	95
4.0	191	(15)*	83	196	(23)	84	232	(3)	104	226	(5)	97
8.0	170	(15)†	74	192	(13)	82	235	(2)	106	228	(4)	98
16.0	ND			ND			224	(6)	101	228	(4)	98
32.0	ND			ND			231	(4)	104	212	(7)‡	91
p value§	0.002			0.104			0.250			0.050		

Note: SEM = standard error of the mean, ND = not done.
*Significantly different from zero dose ($p = 0.04$).
†Significantly different from zero dose ($p = 0.001$).
‡Significantly different from zero dose ($p = 0.05$).
§*F*-test.

Effects of MAO inhibitors in combination with clonidine

TCP and FTCP (0.5, 2 and 8 mg/kg) were administered 15 minutes after clonidine (0.06 mg/kg) and 30 minutes before testing. Clonidine induced additive effects with the parent drug TCP and the analogue FTCP (Table 2). The period of immobility was significantly less for the combination of clonidine and TCP than for the vehicle alone ($p < 0.01$) for the 3 doses of TCP tested and significantly less than TCP alone at 2 mg/kg ($p < 0.01$). FTCP displayed a similar profile when administered with clonidine, and the period of immobility was much less for the combination than for FTCP alone ($p < 0.001$ for the 3 doses of FTCP tested).

The administration of clonidine (0.06 mg/kg) induced a strong additive effect with both PLZ and AcPLZ as well. The period of immobility was significantly less for the combination of clonidine and PLZ than for the vehicle alone ($p < 0.01$) and was also less than for PLZ alone at doses of 8 and 32 mg/kg ($p < 0.01$). AcPLZ displayed a similar profile when administered with clonidine, and the period of immobility was shorter for all tested doses ($p < 0.001$).

Effects of MAO inhibitors in combination with lithium

Pretreatment with lithium (1 mEq/kg) did not modify the effects of any of the MAO inhibitors (Table 3).

Effects of MAO inhibitors in combination with quinine

The administration of quinine (0.5 mg/kg) did not induce additive effects with TCP, FTCP or PLZ (Table 4), but quinine did have additive anti-immobility effects with AcPLZ ($p < 0.01$ for doses of 2 and 32 mg/kg, and $p < 0.05$ for dose of 8 mg/kg).

Table 2: Effect of clonidine on anti-immobility effects of the 4 MAO inhibitors

MAO inhibitor; dose, mg/kg	Treatment; mean period of immobility (and SEM), s				% change†	*p* value‡
	Single drug*		Interaction (clonidine + MAO inhibitor)			
TCP						< 0.001
0.0	188	(21)	NA		NA	
0.5	204	(12)	163§	(19)	–17	
2.0	214	(12)	133§¶	(25)	–35	
8.0	170§	(15)	102§	(25)	–30	
FTCP						< 0.001
0.0	230	(2)	NA		NA	
0.5	228	(3)	191¶	(6)	–16	
2.0	208	(17)	173§¶	(11)	–15	
8.0	191	(13)	129§¶	(22)	–27	
PLZ						< 0.001
0.0	214	(6)	NA		NA	
2.0	223	(5)	170	(22)	–23	
8.0	235	(2)	143§¶	(21)	–42	
32.0	231	(3)	170¶	(13)	–27	
AcPLZ						< 0.001
0.0	214	(6)	NA		NA	
2.0	219	(10)	155§¶	(20)	–27	
8.0	228	(4)	182	(11)	–20	
32.0	211	(7)	111§¶	(26)	–43	

Note: TCP = tranylcypromine, FTCP = 4-fluorotranylcypromine, PLZ = phenelzine, AcPLZ = N^2-acetylphenelzine, NA = not applicable.
*For zero doses of the 4 MAO inhibitors, the data are for clonidine alone (0.06 mg/kg).
†Calculated as (% interaction – % MAO inhibitor alone), where the percentages are calculated with respect to mean periods of immobility (and SEM) for control (vehicle). Mean period of immobility (and SEM) for controls, in seconds: TCP 231 (3), FTCP 223 (2), PLZ 222 (5) and AcPLZ 232 (3).
‡*F*-test.
§Significantly different from vehicle ($p < 0.01$) (Sidak's test, $n = 10$).
¶Significantly different from MAO inhibitor alone ($p < 0.01$) (Sidak's test, $n = 10$).

Discussion

The classic biogenic amine theory of depression is based in part on the antidepressant action of MAO inhibitors and monoamine reuptake blockers. Initially, a functional deficiency of noradrenaline or 5-hydroxytryptamine (5-HT) in the synaptic cleft was proposed as the neuronal basis of depression. However, this theory fails to explain some basic aspects of depression, since some antidepressants do not appear to increase the synaptic concentration of monoamines. A growing body of evidence suggests that the therapeutic activity of antidepressants may involve direct action on several receptor systems. The FST has been described as particularly sensitive to drugs that enhance noradrenergic transmission,[19] and the 5-HT system has also been implicated.[16–18,20]

The behavioural results of the present FST study indicate that when administered alone, the MAO inhibitors TCP and PLZ and their analogues, FTCP and AcPLZ respectively, were poorly active or without any effect. These results agree with previous results obtained in our laboratory: all previously studied MAO inhibitors, including moclobemide (a reversible inhibitor of MAO-A), pargyline (an inhibitor of MAO-B at low doses and of MAO-A and MAO-B at higher doses), nialamide (a mixed MAO inhibitor) and Ro 16-6491 (an inhibitor of MAO-B),[15–17] were inactive in the FST when administered alone.

When combined with clonidine,[15,20] lithium[16,21] or quinine[17] several antidepressants given at doses that were subactive when the drugs were administered on their own produced significant anti-immobility effects in the FST. Clonidine administered at a very low dose (0.06 mg/kg) rendered all classes of antidepressants tested active;[14] activation by lithium and quinine appeared to be more selective.[18]

In the present study clonidine clearly potentiated the anti-immobility effects of all 4 MAO inhibitors tested. Serotonergic neurotransmission is known to be in-

Table 3: Effect of lithium on the anti-immobility effects of the 4 MAO inhibitors

MAO inhibitor; dose, mg/kg	Treatment; mean period of immobility (and SEM), s				% change†
	Single drug*		Interaction (lithium + MAO inhibitor)		
TCP					
0.0	218	(4)	NA		
0.5	223	(4)	192	(7)	–14
2.0	189	(6)	183	(6)	–3
8.0	155‡	(14)	165‡	(15)	+4
FTCP					
0.0	230	(3)	NA		
0.5	225	(5)	206	(5)	–8
2.0	220	(6)	193§	(10)	–12
8.0	209	(5)	206	(8)	–1
PLZ					
0.0	223	(8)	NA		
2.0	227	(4)	224	(5)	–2
8.0	228	(4)	223	(4)	–2
32.0	204‡	(6)	213	(3)	+4
AcPLZ					
0.0	227	(3)	NA		
2.0	226	(2)	221	(4)	–2
8.0	226	(4)	226	(3)	0
32.0	207‡	(6)	204‡	(7)	–1

*For zero doses of the 4 MAO inhibitors, the data are for lithium alone (1 mEq/kg).
†Calculated as (% interaction – % MAO inhibitor alone), where the percentages are calculated with respect to mean periods of immobility for control (vehicle). Mean period of immobility (and SEM) for controls, in seconds: TCP 216 (4), FTCP 224 (4), PLZ 228 (4), AcPLZ 231 (1).
‡Significantly different from vehicle ($p < 0.01$) (Sidak's test, $n = 10$).
§Significantly different from vehicle ($p < 0.05$) (Sidak's test, $n = 10$).

Table 4: Effect of quinine on the anti-immobility effects of the 4 MAO inhibitors

MAO inhibitor; dose, mg/kg	Treatment; mean period of immobility (and SEM), s				% change†
	Single drug*		Interaction (quinine + MAO inhibitor)		
TCP					
0.0	217	(4)	NA		
0.5	223	(4)	206	(5)	–8
2.0	189	(6)	187	(9)	–1
8.0	155‡	(14)	178‡	(10)	+10
FTCP					
0.0	227	(3)	NA		
0.5	235	(9)	221	(18)	–6
2.0	236	(6)	234	(14)	–1
8.0	222	(23)	216	(22)	–3
PLZ					
0.0	231	(3)	NA		
2.0	232	(2)	225	(4)	–3
8.0	228	(3)	223	(4)	–2
32.0	227	(4)	226	(4)	0
AcPLZ					
0.0	221	(5)	NA		
2.0	227	(3)	194‡	(5)	–15§
8.0	221	(4)	196‡	(9)	–11¶
32.0	223	(3)	178‡	(6)	–21§

*For zero doses of the 4 MAO inhibitors, the data are for quinine alone (0.5 mg/kg).
†Calculated as (% interaction – % MAO inhibitor alone), where the percentages are calculated with respect to mean periods of immobility for control (vehicle). Mean period of immobility (and SEM) for controls, in seconds: TCP 216 (4), FTCP 231 (2), PLZ 231 (3), AcPLZ 221 (5).
‡Significantly different from vehicle ($p < 0.01$) (Sidak's test, $n = 10$).
§Significantly different from AcPLZ alone ($p < 0.01$) (Sidak's test, $n = 10$).
¶Significantly different from AcPLZ alone ($p < 0.05$) (Sidak's test, $n = 10$).

creased through attenuation of the release of endogenous noradrenaline, through activation of α_2-adrenergic autoreceptors on noradrenergic neurones,[22] and this effect may account for the additive activity of clonidine in the FST. Clonidine also induces anti-immobility effects with the 5-$HT_{2A/2C}$ receptor antagonist ritanserin in the mouse FST[23] and the mouse tail suspension test.[18] In fact, in a study investigating the possible influence of central 5-HT function on clonidine-induced hypoactivity, the 5-$HT_{2A/2C}$ receptor antagonists ritanserin and ketanserin both potentiated clonidine-induced hypoactivity in a dose-dependent manner.[24] It was concluded that the additive effects of clonidine with antidepressant drugs may be due in part to action at specific serotonergic receptor subtypes and not just to α_2-adrenoreceptor activation. Clonidine was tested in combination with selective agonists and antagonists at 5-HT_1 and 5-HT_2 receptor subtypes, and it was concluded that its anti-immobility effects are more likely mediated by 5-HT_{1A} and 5-HT_{2C} receptors.[20] 5-HT_2 receptors are down-regulated after long-term treatment with several types of antidepressants,[25] and 5-HT_2 antagonists have been reported to have antidepressant-like effects in the clinical setting.[26] TCP is known to down-regulate 5-HT_2 receptors,[27] and higher doses of this drug cause significantly greater inhibition of MAO-A and MAO-B, higher levels of 5-HT and greater down-regulation of 5-HT_2 receptors than low doses. These findings are in line with the results of the present study, whereby the potentiating effect of clonidine was more effective for the higher doses (4 and 8 mg/kg) of TCP. The additive effect seemed more pronounced with the analogue, FTCP. PLZ is also known to down-regulate 5-HT_2 receptors in rat brains after long-term administration.[28]

Neither the parent drugs, TCP and PLZ, nor the analogues, FTCP and AcPLZ respectively, induced anti-immobility effects in the FST when given in combination with lithium. In a review of the effects of acute administration of lithium and different classes of MAO inhibitors, the irreversible MAO-A inhibitor moclobemide was distinct from the other MAO inhibitors reviewed by virtue of its significant anti-immobility effect when combined with lithium.[16] MAO-A preferentially deaminates 5-HT and NA.[29,30] A reversible inhibitor of MAO-B, Ro 16-6491, is a metabolite of moclobemide in rodents but not in humans;[31] it is inactive in the FST alone and in combination with lithium.[16] The absence of any potentiation by lithium of nonselective MAO inhibitors in this study agrees with our previous findings.[16] The reasons for the activity of moclobemide in this test and the lack of effect with the nonselective MAO inhibitors are not yet clear, given that all of these MAO inhibitors inhibit MAO-A. However, other studies have demonstrated that prior administration of lithium potentiates the anti-immobility effects of the 5-$HT_{1A/B}$ receptor agonist RU 24969, as well as those of the more specific 5-HT_{1B} receptor agonist anpirtoline.[32] These results suggest that the 5-HT_{1B} receptor may play a major role in lithium additive effects in the mouse FST.[21]

Of the 4 MAO inhibitors tested in combination with quinine, AcPLZ was the only one that produced anti-immobility effects at the doses tested. In a previous study,[17] subactive doses of moclobemide and pargyline produced anti-immobility effects in mice that had been pretreated with quinine. The MAO-B inhibitor Ro 16-6491 lacked an effect. The reasons for the differences among the various MAO inhibitors in this test are not yet clear. Quinine also potentiates the effect of the 5HT3 receptor antagonist ondansetron.[23] The 5-HT_3 receptor is directly coupled to an ion channel that is highly permeable to sodium and potassium ions.[33] Quinine is regarded as a calcium-activated potassium channel blocker.[34] Such blockade prolongs neuronal activity, which leads to increased presynaptic release and hence synaptic availability of neurotransmitter. Another possible contributor to quinine's effects could result from metabolism. Specifically, quinine is a known inhibitor of the enzyme cytochrome *P*-450 2D6.[35] It is this enzyme that is responsible for the deactivation, usually through hydroxylation, of many compounds, including antidepressants. However, there is a paucity of reports on such interactions between quinine and MAO inhibitors. Both TCP and PLZ are inhibitors of oxidative microsomal reactions though an interaction with various cytochrome *P*-450 enzymes.[1]

In conclusion, TCP, PLZ and their analogues did not demonstrate effects in the FST when administered alone. However, all 4 drugs were active in combination with clonidine, perhaps through mixed activity at 5-HT_{1A} and 5-HT_2 receptors and the noradrenergic system. 5-HT_{1B} receptors do not seem to be implicated, as lithium did not potentiate the effect of any of the drugs. Quinine activation of AcPLZ may indicate that this analogue acts on 5-HT_3 receptors. The findings from this behavioural study thus support previous neurochemical findings suggesting that FTCP and AcPLZ have antidepressant properties. AcPLZ may

also have some advantages over PLZ. It is noteworthy that preliminary studies have demonstrated neuroprotective effects of AcPLZ in mouse brain ex vivo.[36] This observation is particularly interesting given recent reports[37,38] that antidepressants can positively regulate neuroprotective genes.

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