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Although Chemically Related to Amineptine, the Antidepressant Tianeptine Is Not a Dopamine Uptake Inhibitor

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VAUGEOIS, J.-M., A. T. CORERA, A. DESLANDES AND J. COSTENTIN. *Although chemically related to amineptine, the antidepressant tianeptine is not a dopamine uptake inhibitor.* PHARMACOL BIOCHEM BEHAV **63**(2) 285–290, 1999.—We investigated whether the antidepressant tianeptine shares the dopamine uptake inhibitory properties of the chemically related antidepressant amineptine. Tianeptine dose dependently (5, 10, 20, 40 mg/kg IP) increased locomotor activity in mice. This stimulant effect (20 mg/kg IP) was dose dependently prevented not only by the D_1 dopamine receptor antagonist SCH 23390 (7.5, 15, 30 μg/kg SC), but also by the D_2 dopamine receptor antagonist haloperidol (50, 100, 200 μg/kg IP), in contrast to that elicited by dopamine uptake inhibitors. Where the latter prevent dexamphetamine-induced (3 mg/kg SC) reversion of akinesia in mice pretreated with reserpine (4 mg/kg SC, 5 h before test), tianeptine (20 mg/kg IP, 30 min before test) did not. Tested up to a concentration of 10^{-4} M, tianeptine did neither inhibit the [^{3}H]dopamine uptake into mouse striatal synaptosomes nor compete in vitro with the specific binding of [^{3}H]WIN 35,428 at dopamine transporters from striatal membranes. Finally, in mice injected IV with a tracer dose of [^{3}H]WIN 35,428 (1 μCi), the highest tested dose of tianeptine (40 mg/kg IP) did not reduce the specific binding of the radioligand to striatal dopamine transporters. It is concluded that the antidepressant effect of tianeptine does not depend upon a blockade of the neuronal dopamine transporter. © 1999 Elsevier Science Inc.

Tianeptine Dopamine uptake inhibition Locomotion Mouse [^{3}H]WIN 35,428 binding

THE efficacy and safety of tianeptine vs. antidepressant reference compounds have been demonstrated for major depression and dysthymia in numerous clinical studies (7,8). Tianeptine is also active in a number of animal antidepressant screening models such as learned helplessness (27), the olfactory bulbectomized rat (16), and the Porsolt despair test (22). Furthermore, tianeptine reduces stimulation of the hypothalamic–pituitary–adrenal axis in response to stress, and antagonizes stress-induced behavioral deficits (9,30). It inhibits reserpine and tetrabenazine ptosis in rats at relatively high doses (20–40 mg/kg IP), while at lower doses (10 mg/kg IP) it blocks immobility in the Porsolt despair test (22).

Tianeptine is considered as a midposition antidepressant according to therapeutic classification (18). Its therapeutic profile appears to be neither stimulating nor sedative. Tianeptine is an atypical tricyclic molecule with a substituted dibenzothiazepine nucleus containing two heteroatoms, and a long lateral amino-heptanoic chain with a terminal acidic group. Its chemical structure resembles that of the older antidepressant amineptine. Amineptine has a dibenzosuberan nucleus on which has been grafted a similar lateral 7-aminoheptanoic chain (Fig. 1).

Amineptine is a dopamine uptake blocker both in vitro and in vivo (6,28). Controlled trials have generally shown

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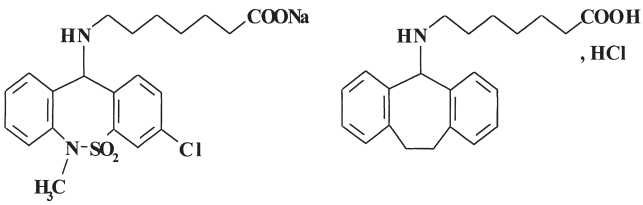

FIG. 1. Chemical structures of tianeptine and amineptine.

equal efficacy of amineptine with reference to antidepressants at an end point. However, in comparison with other tricyclics and fluoxetine, amineptine exerts a psychostimulant effect from the beginning of the treatment (5).

The aim of this work was to check, using several behavioral and neurochemical tests, whether tianeptine interacts in vitro and in vivo with the neuronal dopamine transporter as it is the case for amineptine and other antidepressants like nomifensine or bupropion.

METHOD

Animals

Male Swiss albino CD1 mice, weighing 21–23 g, were purchased from Charles River (Saint Aubin lès Elbeuf, France). They were housed by 30 in Makrolon cages (38 × 24 × 18 cm) with free access to water and food (U.A.R., France) and kept in a ventilated room at a temperature of 21 ± 1°C, under a 12-h light:dark cycle (light on between 0700 and 1900 h). Experiments were carried out between 0900 and 1900 h.

Testing Procedures

Procedures used in this study are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Locomotor activity. Locomotor activity was measured with a Digiscan actometer (Omnitech Electronics Inc., Columbus, OH), which monitored the horizontal movements of the animals. The individual compartments (L = 20; W = 20; H = 30 cm) were put in a dimly lit and quiet room. The responses to drugs injected before the test were expressed as number of beams crossed during 45 min (dose–response study) or 30 min (other studies).

In vitro uptake of [^{3}H]dopamine. A crude synaptosomal fraction (S1) was obtained by homogenization (Potter-Elvehjem, clearance 80–130 μm) of the mouse striatum in 10 vol of ice-cold 0.32 M sucrose containing pargyline (0.1 mM) followed by centrifugation (1000 × *g*, 10 min, 4°C). Aliquotes of the supernatant (50 μl) were preincubated (37°C) with 900 μl of modified Krebs-Ringer phosphate buffer (NaCl 109 mM, $MgSO_4$ 1 mM, KH_2PO_4 1 mM, $NaHCO_3$ 27 mM, glucose 5.4 mM; pH 7.4 ± 0.1). Then, [^{3}H]dopamine, at a 20 nM final concentration (50 μl), was added for a 5-min incubation period. Uptake was stopped by dilution with ice-cold Krebs-Ringer phosphate buffer (4 ml) and immediate centrifugation (7,000 × *g*, 10 min, 4°C). The pellet was sonicated (microprobe diameter 3 mm; Sonics & Materials, Danbury, CT) in 250 μl distilled water, and aliquotes of the homogenate were used for the determination of radioactivity and protein concentration. The radioactivity was determined by liquid scintillation spectrometry (Betamatic V, Kontron, Trappes France) in 4 ml UltimaGold® (Packard, France) with a 30–37% counting efficiency. The protein concentration was determined by the method of Lowry (19) using bovine serum albumin as a standard. The specific uptake of [^{3}H]dopamine was defined as the difference between the total uptake at 37°C and the nonspecific accumulation at 0°C.

In vitro binding of [^{3}H]WIN 35,428. A crude synaptosomal fraction (S1) was obtained by homogenization (Potter-Elvehjem, clearance 80–130 μm) of the mouse striatum in 15 vol of ice-cold 30 mM $NaHCO_3$/Na H_2PO_4 buffer (29/1) containing 240 mM sucrose (pH 7.7 ± 0.1), followed by centrifugation (1,000 × *g*, 10 min, 4°C). The supernatant was recentrifuged (17,000 × *g*, 20 min, 4°C).The resultant pellet was resuspended in 15 vol of buffer by sonication for 5 s (microprobe diameter 3 mm; Sonics & Materials). The protein concentration was determined by the method of Lowry (19). The binding was carried out in a total volume of 0.5 ml containing [^{3}H]WIN 35,428 (1 nM final concentration) drug or buffer and membranes (50 μg per tube). The incubation period (2 h at 4°C) was stopped by rapid vacuum filtration through GF/B filters previously soaked for at least 1 h in 0.5% polyethyleneimine. Each tube was rinsed once; the filters were washed four times with 5 ml of ice-cold buffer and the radioactivity counted by liquid scintillation spectrometry (Betamatic V, Kontron, Trappes, France) in 4 ml UltimaGold® (Packard, France) with a 30–37% counting efficiency. The specific binding was calculated by subtracting the nonspecific binding defined in the presence of 10 μM mazindol from the total binding.

In vivo binding of [^{3}H]WIN 35,428. [^{3}H]WIN 35,428 (1 μCi in 200 μl saline containing 0.5% ethanol) was injected via a tail vein, and the mice were decapitated 45 min later. Their brains were rapidly removed and the striatum and cerebellum were dissected out on ice. These regions were sonicated in 0.9% saline (2 ml for striatum, 5 ml for cerebellum). A 750-μl sample of each homogenate was counted in a minivial containing 4 ml Ultimagold® (Packard, France) by liquid scintillation spectrometry (Betamatic V, Kontron, Trappes, France). Protein concentrations were determined in a 20-μl sample of each homogenate according to the method of Lowry (19). Results are expressed in fmol/mg of protein. After determining radioactivity content, striatal-to-cerebellar (striatum/cerebellum) ratios of radioactivity were calculated. These ratios would be decreased by drugs that bind to dopamine transporters located on dopaminergic terminals, which are highly concentrated in the striatum and absent in the cerebellum.

Drugs

[^{3}H]dopamine (12–12.8 Ci/mmol) was purchased from Amersham (Les Ulis, France). [^{3}H]WIN 35,428 (83.5 Ci/mmol) was purchased from NEN (Les Ulis, France). Mazindol was obtained from Sandoz. Tianeptine (sodium salt), a generous gift from IRIS (Courbevoie, France), was dissolved in saline and intraperitoneally injected. Dexamphetamine sulphate (La Cooper, Melun, France) was dissolved in saline and subcutaneously injected. Haloperidol (Haldol®, Janssen, France) was also diluted in saline to get the appropriate doses, and IP administered. R(+) SCH 23390 HCl (RBI, France) was dissolved in distilled water and SC injected. Reserpine (Sigma, France) was dissolved in dimethyl sulfoxide (Sigma, France) and then diluted in Cremophor EL (Sigma, France) and distilled water (final concentration: 5% DMSO and 5% Cremophor EL) and SC injected. All drugs were prepared fresh daily

and injected in a volume of 10 ml/kg. Doses always refer to the free bases.

Statistics

Results are expressed as means ± SEM. The data from the binding studies were analyzed by Student's *t*-test, and those from the locomotor activity dose–response experiment by Dunnett's *t*-test. A two-way analysis of variance (ANOVA) was used to assess the overall significance of the locomotor activity results when two treatments were administered. When the two-way ANOVA revealed an interaction between factors, it was followed by separate one-way ANOVAs that focussed on the analysis of tianeptine effects. A minimum acceptable level of significance was set at $p < 0.05$.

RESULTS

Locomotor Activity

In a dose–response study, performed using an automated Digiscan actometer, the administration of tianeptine enhanced horizontal locomotor activity at two doses: 20 and 40 mg/kg (IP) (Fig. 2).

These doses were, therefore, chosen in the next experiments. The locomotor stimulant effect induced by tianeptine (20 mg/kg IP) was dose dependently suppressed in mice pretreated IP with increasing doses of haloperidol. A two-way ANOVA revealed a significant interaction between the treatments haloperidol and tianeptine, $F(3, 56) = 6.49$, $p < 0.001$. Separate one-way ANOVAs were performed for each haloperidol dose. A significant effect of tianeptine was found, $F(1, 14) = 7.61$, $p < 0.02$, in groups of mice pretreated with the vehicle of haloperidol. Tianeptine still produced a significant increase in locomotor activity in haloperidol-treated (50 μg/kg) mice compared with saline control mice, but failed to induce changes, $F(1, 14) = 0.98$, $p > 0.05$, in mice treated with a 100 μg/kg dose of the D_2 dopamine receptor antagonist (Fig. 3, upper panel). The locomotor stimulant effect induced by tianeptine (20 mg/kg IP) was also dose dependently suppressed in mice pretreated SC with increasing doses of SCH 23390. A two-way ANOVA revealed a significant interaction between the treatments SCH 23390 and tianeptine, $F(3, 56) = 6.55$, $p < 0.001$. Separate one-way ANOVAs were performed for each SCH 23390 dose. A significant effect of tianeptine was found, $F(1, 14) = 9.01$, $p = 0.01$, in groups of mice pretreated with the vehicle of SCH 23390. Tianeptine still produced a significant increase in locomotor activity in SCH 23390-treated (7.5–15 μg/kg) mice compared with saline control mice (Fig. 3, lower panel). However, the locomotor activity in tianeptine-treated mice was even decreased with respect to vehicle-treated group, $F(1, 14) = 7.63$, $p < 0.05$, at the highest tested dose of SCH 23390 (Fig. 3, lower panel).

In reserpine-pretreated mice (4 mg/kg SC, 5 h before testing), a significant main effect of dexamphetamine treatment was revealed, $F(1, 36) = 81.52$, $p < 0.001$. Additionally, the stimulant effect of tianeptine (20 mg/kg IP) was abolished in reserpine-pretreated mice as indicated by ANOVA, $F(1, 36) = 0.81$, $p > 0.05$. Furthermore, no interaction was found between the two treatments, indicating that tianeptine did not prevent the reversion of akinesia elicited by dexamphetamine (3 mg/kg SC) in reserpine-pretreated mice (Fig. 4).

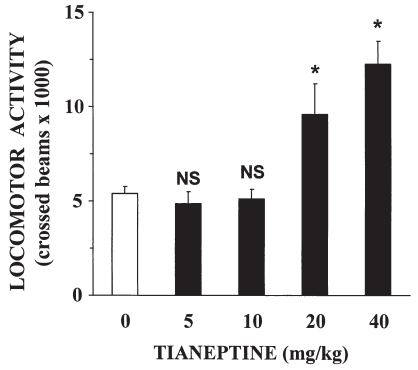

FIG. 2. Effects of increasing doses of tianeptine on locomotor activity. Mice were injected with saline (open columns) or increasing doses of tianeptine (5, 10, 20, 40 mg/kg IP) and were introduced into the actometers. The horizontal activity was measured for 45 min. Means ± SEM of data from 20 controls and 10 mice in treated groups. NS: $p > 0.05$, $*p < 0.01$ (Dunnett's *t*-test) compared to saline.

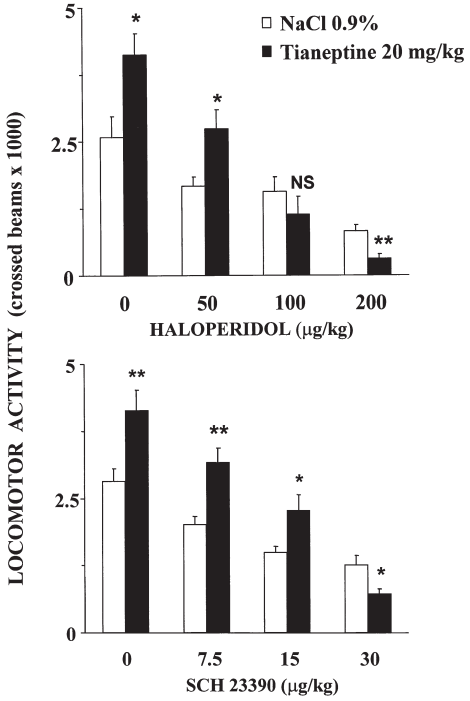

FIG. 3. Effects of haloperidol or SCH 23390 on stimulation of locomotor activity induced by tianeptine. Mice were injected with vehicles (open columns) or increasing doses of either haloperidol (50, 100, 200 μg/kg IP) (hatched columns, upper panel) or SCH 23390 (7.5, 15, 30 μg/kg SC) (hatched columns, lower panel). Fifteen minutes later they were injected with saline or tianeptine (20 mg/kg IP). Fifteen minutes after the second treatment mice were introduced into the actometers. The horizontal activity was measured for 30 min. Means ± SEM of data from eight mice per group. Two-way ANOVAs: (interaction of haloperidol × tianeptine): $F(3, 56) = 6.49$, $p < 0.001$; (interaction of SCH 23390 × tianeptine): $F(3, 56) = 6.55$, $p < 0.001$. Separate one-way ANOVAs: NS: $p > 0.05$, $*p < 0.05$, $**p < 0.01$ compared with haloperidol (same dose)-saline group or with SCH 23390 (same dose)-saline group.

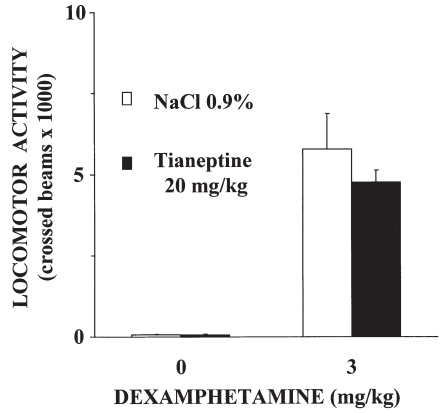

FIG. 4. Effects of reserpine (4 mg/kg SC) or dexamphetamine (3 mg/kg SC) on stimulation of locomotor activity induced by tianeptine (20 mg/kg IP). Four groups of mice were injected with reserpine 5 h before testing. Tianeptine was administered 30 min before testing to two groups and saline to two other groups. Dexamphetamine or its vehicle were injected to appropriate groups just before testing. The horizontal activity was measured for 30 min. Means ± SEM of data from 10 mice per group. A two-way ANOVA revealed no interaction dexamphetamine × tianeptine, $F(1, 36) = 0.80$, $p > 0.05$, a main effect of dexamphetamine, $F(1, 36) = 81.52$, $p < 0.001$, and no main effect of tianeptine, $F(1, 36) = 0.81$, $p > 0.05$.

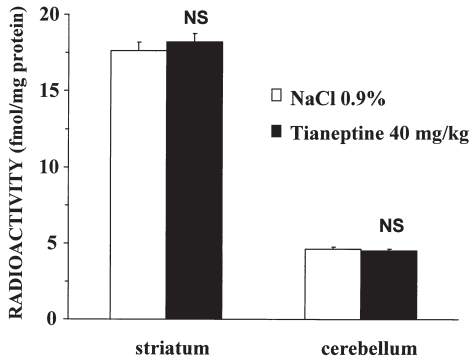

FIG. 5. Effect of a treatment with tianeptine on the striatal and cerebellar in vivo binding of [^{3}H]WIN 35,428. Mice were injected IP with either saline or tianeptine (40 mg/kg) 15 min after the injection of the radioligand. The IV injection of a tracer dose of the dopamine uptake inhibitor [^{3}H]WIN 35,428 (1 μCi per mouse) occurred 45 min before the sacrifice. Striatum and cerebellum were dissected out and the radioactivity within each structure was measured. Means ± SEM of data from eight mice per group. NS: $p > 0.05$ compared to saline controls.

In Vitro Uptake of [^{3}H]Dopamine

Tianeptine, at concentrations between 1 and 100 μM, did not affect uptake of tritiated dopamine by synaptosomes obtained from mouse striatum (1.6 ± 1.8% increase at 100 μM, mean ± SEM of four experiments carried out in duplicate).

In Vitro Binding of [^{3}H]WIN 35,428

Up to the 100 μM concentration, tianeptine did not inhibit the in vitro binding of [^{3}H]WIN 35,428 to neuronal dopamine transporters (0.7 ± 2% increase at 100 μM, mean ± SEM of four experiments carried out in duplicate).

In Vivo Binding of [^{3}H]WIN 35,428

The effects of a high dose of tianeptine on the binding of [^{3}H]WIN 35,428 were studied in the striatum, a region of the mouse brain rich in dopamine transporter, and in the cerebellum, a dopamine transporter-poor site. In this study, the high dose of tianeptine (40 mg/kg IP), injected 15 min after tracer administration of [^{3}H]WIN 35,428, did not inhibit its in vivo binding in the striatum, as shown by the lack of effect upon striatum/cerebellum ratios (controls: s/c = 3.81 ± 0.09 vs. tianeptine-treated mice: s/c = 4.04 ± 0.16). In fact, both striatal and cerebellar concentrations of radioactivity remained unaffected by the treatment with tianeptine (Fig. 5).

DISCUSSION

The classical theory of the biochemical foundation of depression hypothesizes a decrease in central synaptic neurotransmission secondary to the deficiency in monoaminergic neurotransmitters, serotonin and/or noradrenaline. The role for a serotonin deficit is supported by several lines of evidence: lower availability of plasma L-tryptophan, the precursor of serotonin, to the brain in depressed patients; induction of depressive symptomatology by L-tryptophan depletion techniques; the relationship between lower L-tryptophan levels and positive response to serotonergic antidepressive treatments; all clinically effective antidepressants increase the amount of monoamines available in the synaptic cleft, by inhibiting reuptake mechanism (tricyclics) or by inhibiting enzymatic catabolism (MAOIs) (2,20).

The major role suggested for serotonin deficiency in this theory led to the development of a large number of compounds intended to increase serotonin neurotransmission, particularly by blocking serotonin reuptake: the selective serotonin reuptake inhibitors (SSRIs) and more recently by blocking both serotonin and noradrenaline reuptake: the serotonin noradrenaline reuptake inhibitors (SNRIs) (2,4). But animal studies on the mechanism of action of tianeptine have revealed that in contrast to classical tricyclic antidepressants, tianeptine stimulates serotonin reuptake in vivo in the rat brain (12,21). Tianeptine does not bind to any of the receptors tested: 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, 5-HT$_2$, 5-HT$_3$, alpha$_1$-, alpha$_2$- and beta-adrenoreceptors, dopamine D$_2$ receptors, GABA, glutamate, benzodiazepine, muscarinic, and histamine receptors, calcium channels, and does not inhibit monoamine oxidase-A and monoamine oxidase-B activity. The paradoxical finding that both tianeptine and SSRIs exhibit antidepressant activity despite apparently antagonistic mechanisms is rather puzzling. Among the interpretations elaborated in order to try to reconcile these data, Ansseau suggested that tianeptine could be effective through other mechanisms than the unique property of activating serotonin reuptake, and particularly through its effect on the output of dopamine in the nucleus accumbens (15,23). The results of the present study are in accordance with this interpretation.

Moderate stimulant locomotor effects have been previously reported for tianeptine both in rats and mice (22). We observed this effect at relatively high doses in mice, because the antidepressant-like effects have been shown from the 5–10 mg/kg doses (16,22). The involvement of a catecholamine mechanism in this stimulant effect might be suggested. In pre-

vious studies, tianeptine moderately increased the extracellular concentration of dopamine in the nucleus accumbens and in the frontal cortex (15,23). These effects may be involved in its antidepressant activity because numerous studies have shown that various antidepressants may also increase dopamine transmission in the mesocorticolimbic system (10,25,26). However, tianeptine does not inhibit in vitro uptake of [^{3}H]dopamine into synaptosomes (present study), at concentrations that can be reached in vivo (21), nor does the antidepressant inhibit in vitro [^{3}H]WIN 35,428 binding to dopamine transporters.

It would be conceivable that the metabolites of tianeptine (21) but not the parent drug could act at dopamine transporters. To test this hypothesis, the method described by Scheffel et al. (24) for the in vivo labeling of dopamine neuronal transporters with [^{3}H]WIN 35,428 was used. In agreement with these authors, highest striatal-to-cerebellar ratios were obtained when the animals were sacrificed 45 min after the injection of the radiolabeled compound. Consequently, this delay was chosen in our study. Tianeptine is active in screening procedures for antidepressants when the tests occur 30 min after injection, and this interval is commonly used in other laboratories (1). In these experimental conditions, tianeptine and its metabolites are unable to compete in vivo with [^{3}H]WIN 35,428 binding to dopamine transporters. All in all, tianeptine does not increase dopamine transmission by a direct effect on the uptake of dopamine.

A simultaneous stimulation of D_1 and D_2 dopamine receptors is required to elicit locomotor stimulation (3). The stimulant locomotor effects induced by tianeptine were completely antagonized by haloperidol, used at doses up to 200 μg/kg—moderate enough to ensure its D_2 dopamine receptor antagonist selectivity. This is a strong argument against an interaction between this antidepressant and the neuronal dopamine transporter. We have previously observed a lack of antagonism between haloperidol and the dopamine uptake inhibitors BTCP [N-(1-(2 benzo[b]thiophenyl) cyclohexyl) piperidine] or GBR 12783 [1[2-(diphenyl methoxy)ethyl] 4[3-(phenyl-2-propenyl)-piperazine]] or oxolinic acid (11,13). Blockade of D_2 dopamine autoreceptors by haloperidol increases the firing rate of dopaminergic neurones, dopamine synthesis (by derepressing the tyrosine hydroxylase activity), and dopamine release from vesicular stores. Taking advantage of this increased dopamine release, a dopamine uptake inhibitor leads to a more marked increase in synaptic dopamine concentration. This marked increase has been evidenced by microdialysis experiments (29). At these high dopamine concentrations, the amine likely competes more efficiently with haloperidol for occupying the postsynaptic D_2 dopamine receptors, preventing the effect of their blockade. This would explain the lack of antagonism of stimulant locomotor effects of dopamine uptake inhibitors by haloperidol.

Because the stimulation of locomotion in naive rodents requires the simultaneous stimulation of D_1 and D_2 receptors (3), the role of D_1 dopamine receptors in the stimulant effects induced by tianeptine was also investigated. The D_1 receptor antagonist SCH 23390 reversed the effects induced by tianeptine in the locomotor activity test, supporting the evidence of an action of this antidepressant upon dopamine transmission.

The effect of a pretreatment with reserpine allows discrimination between amphetamine-like drugs and pure dopamine uptake inhibitors. The alkaloid prevents the storage of monoamines in neuronal vesicles. Therefore, the firing rate of dopamine neurons no longer triggers the dopamine release, leading to akinesia. In reserpine-treated animals, a pure dopamine uptake inhibitor is unable to increase the synaptic concentration of dopamine, which is nil, and thus does not correct the akinesia. On the contrary, the cytosolic pool of dopamine is spared by reserpine treatment and may be released through the dopamine carrier by amphetamine-like agents. Because tianeptine does not reverse the reserpine-induced akinesia, one may exclude for it an amphetaminic activity. To reverse the reserpine-induced akinesia, amphetaminics must be taken up into dopaminergic neurons, through the dopamine carrier, and promote a release, through the dopamine carrier, of the newly synthesized cytosolic pool of dopamine. Such an effect will be prevented by a pure dopamine uptake inhibitor because it will prevent both the neuronal internalization of amphetaminics and the carrier-mediated release of dopamine, as previously demonstrated for other drugs (14). The lack of prevention by tianeptine of the reversion by amphetamine of the reserpine-induced akinesia therefore argues against a blockade of dopamine carrier.

Finally, at the 40 mg/kg dose inducing a clear stimulation of locomotion, tianeptine did not occupy the dopamine neuronal carriers in the striatum, as evidenced by its lack of effect upon the striatal binding of [^{3}H]WIN 35,428. Although the in vivo occupancy of striatal dopamine uptake complex does not predict the intensity of the stimulant locomotor effect of dopamine uptake inhibitors (28), the complete ineffectiveness of tianeptine in the [^{3}H]WIN 35,428 in vivo assay is another proof of the absence of interaction of this antidepressant with the dopamine uptake complex.

In conclusion, the present work shows that the effects mediated by tianeptine upon the dopaminergic transmissions do not correspond to that of an indirect dopamine agonist such as dopamine uptake inhibitors or amphetamine-like drugs. We further confirm that highly specific structural requirements fundamentally distinguish the structure–activity relationships of the tianeptine series from those of both the classical tricyclic series and the amineptine series (17). However, an indirect facilitating action on dopaminergic transmission in the brain is demonstrated by the reversion of its stimulant locomotor effects by the D_2 receptor antagonist haloperidol. Therefore, an enhanced dopaminergic transmission may play a role in the antidepressant efficacy of tianeptine, although it is devoid of amphetamine-like activity and does not interact with the dopamine neuronal carrier in vitro or in vivo.

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