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DIFFERENTIAL INTERACTIONS OF PHENCYCLIDINE WITH TETRABENAZINE AND RESERPINE AFFECTING INTRANEURONAL DOPAMINE*

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Abstract—This study has examined the effects on synaptosomal (P_2) dopamine of interactions of phencyclidine and some other stimulants with tetrabenazine and reserpine. Tetrabenazine and reserpine both enhanced the spontaneous synaptosomal release of [^{14}C]dopamine and inhibited its formation from [^{14}C]phenylalanine. The [^{14}C]dopamine formation increases induced by phencyclidine and amfonelic acid, however, were affected differentially by coadditions of tetrabenazine and reserpine. At the lower concentrations, tetrabenazine either did not affect or augmented the dopamine formation enhancements by the stimulants. Reserpine at all levels blocked the synthesis enhancements and revealed inhibitory effects of phencyclidine and amfonelic acid upon dopamine formation; only at the highest concentration did the action of tetrabenazine mimick that of reserpine. Amphetamine stimulation of dopamine formation was affected by tetrabenazine and reserpine alike; the stimulation was either maintained or enhanced. Ketamine did not affect dopamine formation either by itself, with tetrabenazine, or with reserpine. In summary, tetrabenazine and reserpine affected synaptosomal dopamine formation and release in a comparable manner, but intraneuronal dopaminergic actions of phencyclidine and also of amfonelic acid may be influenced differentially by these two releasing agents.

Phencyclidine (PCP), currently one of the major drugs of abuse, is a nonamphetamine drug with amphetamine-like central stimulant properties. PCP has been found to reactivate schizophrenic psychosis and produce treatment-resistant schizophrenia-like behavior [1–3]; also, such PCP-induced behaviors have often mimicked the natural disorder to the extent that there have been difficulties in differentiating PCP effects from the occurrences of paranoid schizophrenia [4, 5].

Investigations have suggested that PCP affects a number of neuronal systems; that these include dopamine (DA) neurones [6–11] is particularly interesting in view of the current strong evidence for dopaminergic overactivity in schizophrenia [12, 13].

Investigators have employed various brain preparations to examine if PCP has a direct action on DA neurones that could be demonstrated *in vitro*, and the results indicate that this drug may (a) block the uptake and/or increase the release of preformed labeled DA [8, 14–16] and (b) enhance DA formation from tyrosine precursor.

The results [17] from this laboratory indicate that PCP may not only stimulate formation of DA when a brain synaptosomal preparation is incubated with labeled phenylalanine substrate but also, surprisingly, have a distinct inhibitory effect on the same amine formation, an effect revealed if reserpine (RES) is present in the incubation medium. RES is well known for its releasing effects upon brain monoamines although its full spectrums of actions is not known. It is not clearly understood whether the releasing or some presently unknown action of RES may be causally related to the observed inhibitory effect [17] of PCP upon DA formation. Tetrabenzine (RO 1-9569/7, TBZ), which has antipsychotic properties, shares with RES the releasing effects [18–21] on brain monoamines, including DA. To ascertain if the PCP-induced inhibition of DA formation in the presence of RES is associated with the amine release by the latter drug, the present study has examined PCP actions upon synaptosomal DA in the presence of TBZ and compared the effects of TBZ itself on synaptosomal DA with those of RES. For comparison with PCP, some other stimulant drugs have also been tested for their effects on DA in the presence of RES and TBZ. These drugs are amphetamine (AMT), the behaviorally weaker PCP congener ketamine (KE), and amfonelic acid (AA). AA, which is a powerful nonamphetamine central stimulant, also enhances dopamine formation but becomes inhibitory in the presence of RES [22].

MATERIALS AND METHODS

PCP was bought from the Applied Science Division, State College, PA, and KE was supplied by the Warner-Lambert Co., Ann Arbor, MI. AA was a gift from the Sterling–Winthrop Research Institute, Rensselaer, NY, and TBZ was a gift from Hoffmann–La Roche, Nutley, NJ. RES (as Serpasil) was from Ciba, Summit, NJ, and *d*-amphetamine was from the Sigma Chemical Co. St. Louis, MO. Labeled phenylalanine (L-phenylalanine[U-^{14}C], specific radioactivity 450–500 mCi/mmole) was from the New England Nuclear Corp. Boston, MA.

The experimental methods have been published before in detail [17]. Briefly, a synaptosomal–

* Some of these results were presented at the annual meeting of the Society for Neuroscience, Los Angeles, CA, 1981.

Table 1. Effects of TBZ and RES on synaptosomal [^{14}C]DA formation and release*

Drug (N)	Concn (μM)	[^{14}C]DA (total %)	Release index
None (18)		100% (301.1 ± 21.6)	1.00 (10.9 ± 0.7)
TBZ (6)	0.022	67.7 ± 6.2	1.39 ± 0.8
RES (8)		52.5 ± 3.3†	2.23 ± 0.16‡
TBZ (8)	0.09	48.0 ± 2.3§	2.07 ± 0.11§
RES (7)		42.9 ± 2.4§	2.46 ± 0.16§
TBZ (8)	0.36	31.1 ± 1.5§	3.48 ± 0.14§
RES (10)		32.1 ± 1.3§	3.78 ± 0.21§
TBZ (8)	1.80	25.6 ± 1.4§	5.06 ± 0.52§
RES (15)		25.0 ± 1.2§	5.30 ± 0.19§

*The standard incubation mixture contained the P_2 fraction from 9.5 mg caudate tissue suspended in Tris buffer (pH 7.4) containing salts, glucose, sucrose and pargyline in a final volume of 210–230 μl, and the additions were as 10 μl of aqueous solutions (see Materials and Methods). The [^{14}C]phenylalanine substrate concentration and specific radioactivity were 6.1 μM and 209.0 nCi/nmole respectively. After 10 min of incubation (37°) the mixtures were filtered, and separated fractions were analyzed. The release index was calculated by dividing the medium/total ratio of [^{14}C]DA observed in the presence of an addition by the same ratio in the corresponding control (no addition) sample. The results are expressed as means of a number (N) of observations ± S.E.M. All the values of [^{14}C]DA formation and the release index differed significantly from the corresponding control (no addition) value. The uptake of [^{14}C]phenylalanine substrate following various additions of TBZ and RES did not differ significantly from the control uptake of 1754.3 ± 36.9 pmoles per 100 mg per 10 min. The values for the significance of difference between TBZ and RES results are as indicated. The values given in parentheses are: [^{14}C]DA as pmoles per 100 mg per 10 min; the release as medium [^{14}C]DA/total [^{14}C]DA × 100.

†$P < 0.05$.

‡$P < 0.005$.

§ Not significant.

mitochondrial (P_2) preparation from rat brain caudate nuclei was incubated (37°) with labeled phenylalanine in Tris buffer (10 mM, pH 7.4) containing NaCl (125 mM), KCl (5 mM), $MgCl_2$ (1.5 mM), pargyline (0.08 mM), glucose (10 mM) and sucrose (0.32 M). The mixture (210–230 μl) contained P_2 preparation representing 9.5 mg of the original whole caudate tissue weight. The various drug additions were as aqueous solutions to the incubation medium. Immediately after the 10-min incubation, the synaptosomes were quickly (5–10 sec) separated from the medium and washed on a Millipore filter (0.8 μm). The separated particulate and the medium fractions were then extracted with 0.4 N perchloric acid and analyzed on an alumina column for labeled DA which was assayed by liquid scintillation counting. The level of ^{14}C in the effluent and washings from the alumina column during the synaptosomal acid extract analysis indicated the synaptosomal uptake of the labeled substrate. The product formation and substrate uptake were expressed as pmoles (of product/substrate per 100 mg (of whole caudate tissue equivalent of the P_2 sample) per 10 min (of incubation) obtained from the radioactivities of the analyzed fractions and the final specific radioactivity of the labeled substrate in the incubation mixture. The results are expressed as mean ± standard error of the mean (S.E.M.) with the indicated level of significance (P).

The expressed phenylalanine substrate concentrations were based on the sum of endogenous (tissue), labeled and added cold L-phenylalanine present in the final incubation mixture. The determination of tissue levels of phenylalanine was by ion-exchange chromatography and mass fragmentography [17].

RESULTS

The results in Table 1 show the total (the sum of particulate and medium fractions) synthesis of ([^{14}C]DA in the presence of TBZ and RES, and these data are expressed as the percentage of the control (no addition) value of pmoles per 100 mg per 10 min given within parentheses. Table 1 also summarizes the stimulating effects of TBZ and RES on the release of synaptosomally formed [^{14}C]DA; the release index indicates the release above that in the control sample. The index was derived by dividing the medium/total ratio of [^{14}C]DA in the presence of an addition by that ratio from the control, given in parentheses. For these experiments, aliquots of the same P_2 preparation were generally used to test the effects of TBZ and RES at each concentration level.

Total DA formation from [^{14}C]phenylalanine was inhibited by TBZ and RES. At a 0.09, 0.36 or 1.80 μM concentration the inhibition by TBZ did not differ significantly from that induced by RES. At 0.022 μM, TBZ-induced inhibition was slightly weaker than that observed in the presence of RES. The release indices summarized in Table 1 show that both TBZ and RES markedly enhanced the synaptosomal release of [^{14}C]DA. Also, the releasing effect of TBZ at 0.09, 0.36 or 1.80 μM did not differ significantly from the effect of RES at the corresponding levels. At 0.022 μM TBZ, the release index was found to be lower than that at the same RES concentration. The results in Table 1 clearly indicate that any increase in the concentration of TBZ, or of RES, resulted in higher release indices and, in parallel, greater inhibition of [^{14}C]DA formation.

Table 2. TBZ or RES stimulation of synaptosomal [^{14}C]DA release*

Drug	Concn (μM)	[^{14}C]DA release index PCP (9.1 μM)	AMT (9.1 μM)	AA (0.91 μM)	KE (9.1 μM)
TBZ	0.022	2.03 ± 0.37	2.42 ± 0.33	1.86 ± 0.27	
RES		2.33 ± 0.10†	2.65 ± 0.36†	3.24 ± 0.34‡	
TBZ	0.09	1.62 ± 0.18	1.76 ± 0.14	1.22 ± 0.15	1.05 ± 0.01
RES		2.28 ± 0.13†	2.95 ± 0.37§	2.59 ± 0.32‖	1.15†
TBZ	0.36	1.66 ± 0.09	2.02 ± 0.16	1.14 ± 0.10	
RES		1.61 ± 0.13†	2.84 ± 0.16§	2.45 ± 0.18¶	
TBZ	1.80	1.55 ± 0.08	1.63 ± 0.11	1.45 ± 0.06	1.03 ± 0.06
RES		1.77 ± 0.18†	2.02 ± 0.08‡	1.53 ± 0.11†	1.04 ± 0.05†

*The methods of incubation are described in Table 1. PCP, AMT, AA and KE were added to the incubation mixtures to the indicated concentrations in the presence of various medium levels of TBZ and RES. The data are expressed as release indices relative to the corresponding basal medium [^{14}C]DA level in the presence of TBZ or RES only. The number of observations (N) was between 3 and 7. The release indices with TBZ and RES differed significantly as indicated below.

†Not significant.
‡$P < 0.05$.
§$P < 0.025$.
‖$P < 0.01$.
¶$P < 0.005$.

The results in Table 2 indicate the release of [^{14}C]DA following the addition of either PCP, AMT, AA or KE with either TBZ or RES present in the incubation medium; the release indices were determined from the respective basal values in the presence of TBZ or RES. Each of these stimulants increased the medium fraction of [^{14}C]DA at various concentrations of medium TBZ and RES. The PCP-induced increment of release with TBZ in the medium was slightly lower than that observed in the presence of the corresponding concentration of RES. The release indices were also somewhat lower following the coadditions of AMT and TBZ relative to those after the stimulant and RES coadditions. AA was also a weaker releaser with TBZ in the medium, whereas KE was ineffective with any of the coadditions tested.

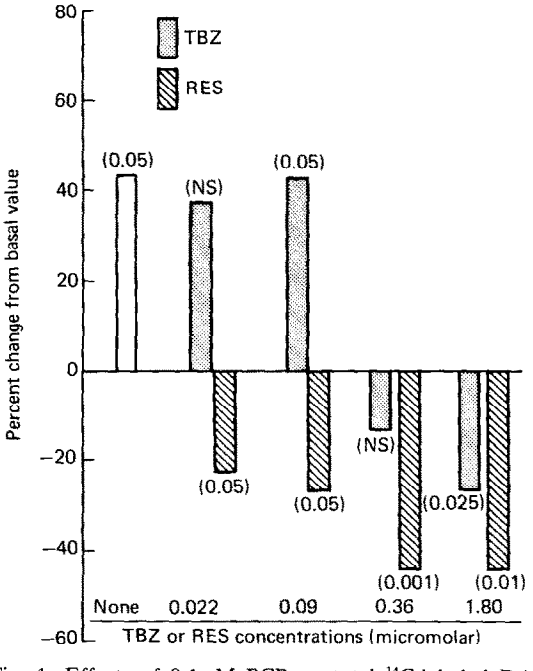

Fig. 1. Effects of 9.1 μM PCP on total ^{14}C-labeled DA formation in the presence of either TBZ or RES at the indicated concentrations. Significance of difference ($P <$) from the corresponding basal value was as indicated, and N varied from 3 to 9. For the methods of incubation see Table 1.

Figures 1–5 summarize the total (particulates plus medium) formation of [^{14}C]DA following the various coadditions of PCP, AMT, AA and KE with either TBZ or RES. The data indicating [^{14}C]DA formations are expressed as the percent change (− for inhibition; + for stimulation) from the basal [^{14}C]DA formations in the presence of TBZ or RES in the medium. In the presence of 0.022 and 0.09 μM TBZ, PCP (9.1 μM) stimulated (Fig. 1) [^{14}C]DA formations above the respective basal values. With a further increase of TBZ concentration to 0.36 μM, PCP weakly (NS) inhibited [^{14}C]DA formation, but at 1.80 μM medium TBZ, PCP was clearly inhibitory. PCP reduced [^{14}C]DA formations below the basal levels in the presence of RES at all levels. AMT (9.1 μM), in contrast to PCP, stimulated (Fig. 2) the labeled DA formation above the basal values at each of the TBZ and RES levels.

AA effects (Fig. 3) were similar to those of PCP when TBZ was present in the incubating medium. AA markedly increased the formation of labeled DA at the lower TBZ concentrations, whereas at 1.80 μM TBZ, AA was inhibiting, although weakly (NS). AA, again like PCP, reduced (Fig. 3) labeled DA formation to levels below the basal values at all the RES concentrations. As with its effects on [^{14}C]DA release, KE was ineffective (Figs. 4 and 5) following any of its coadditions with TBZ or RES.

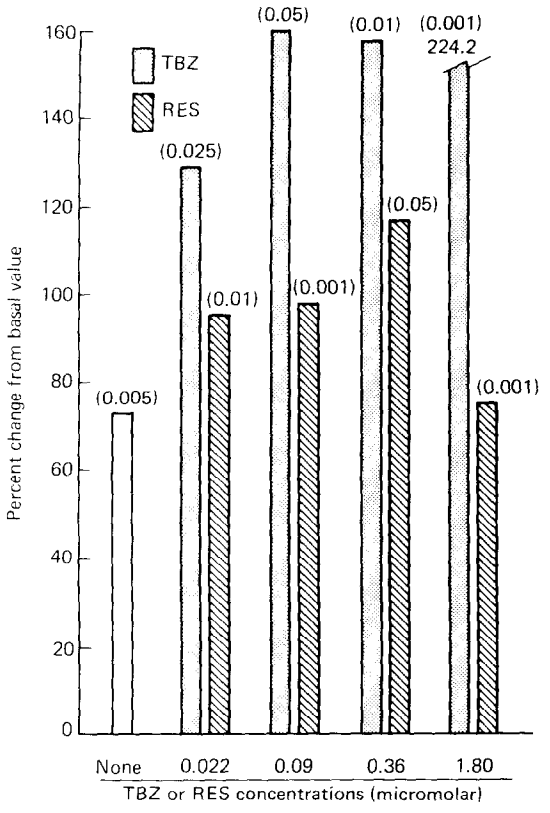

Fig. 2. Effects of 9.1 μM AMT on labeled DA formation in the presence of either TBZ or RES. For other details, see the legend of Fig. 1.

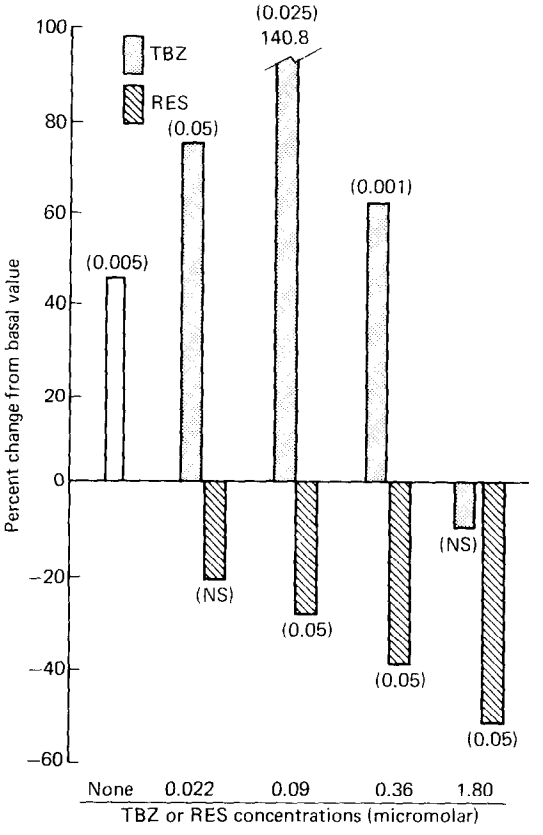

Fig. 3. Effects of 0.91 μM AA on labeled DA formation in the presence of either TBZ or RES. For other details, see the legend of Fig. 1.

For comparative purposes, also included in Figs. 1–5 are the effects of PCP, AMT, AA and KE upon [^{14}C]DA formations in the medium free of TBZ or RES. Compared to the control value in Table 1, the increments of [^{14}C]DA levels were significant in the presence of PCP, AMT and AA but not KE.

DISCUSSION

The results in Table 1 indicate that TBZ shares with RES some of the actions of the latter drug on synaptosomal DA. RES-induced release of continuously appearing DA has been reported before [17, 23], and it is also consistent with the much investigated actions of this drug [24, 25] on catecholamines. TBZ has also been shown to release endogenous brain amines, including DA, following drug action *in vivo* [18–21]. The present results demonstrate that TBZ is also active *in vitro* in releasing DA and that the action of TBZ appears to be rapid (effect observed within 10 min of incubation) and parallel to that of RES. The depletion of catecholamines by RES probably occurs due to the action of this drug on vesicular stored amines [24]; RES inhibition of DA uptake by synaptic vesicles also has been demonstrated [25]. Therefore, any RES addition to the incubation mixture may impair the retention and uptake capacities of the storage vesicles for DA; a higher cytoplasmic amine level would probably be the result, with an inhibitory effect on DA formation in turn. If, indeed, this is the mechanism of the RES-induced inhibition of DA formation (Table 1) demonstrated before [23, 26, 27], the observed inhibitory effect of TBZ on DA formation would suggest similar intraneuronal actions of this drug and RES. Although little is actually known about the intraneuronal actions of TBZ, the fact that this drug

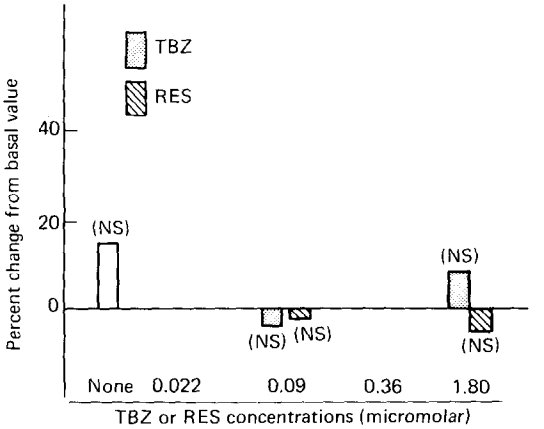

Fig. 4. Effects of 9.1 μM KE on labeled DA formation in the presence of either TBZ or RES. For other details, see the legend of Fig. 1.

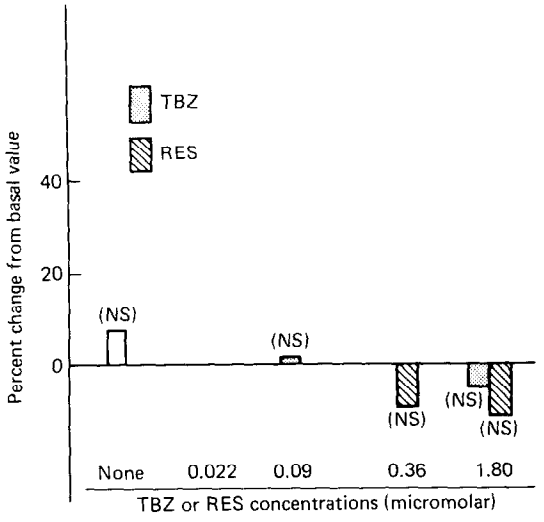

Fig. 5. Effects of 36.4 μM KE on labeled DA formation in the presence of either TBZ or RES. For other details, see the legend of Fig. 1.

(5.0 mg/kg) was as effective as RES (5.0 mg/kg) in causing a near total (> 90%) depletion of striatal DA [21] also suggests similar effects of these two drugs on the vesicles storing DA.

AMT is a well-known releaser of DA and also an inhibitor of amine uptake [28–30]. AMT stimulates synaptosomal formation of DA via hydroxylations of its amino acid precursors [22, 27] in the presence or absence of RES. It is thought that such stimulation is due to a release of the cytoplasmic amine disinhibiting the hydroxylations, while DA formation from labeled dopa is not affected by AMT [31]. The present results show that AMT may release [^{14}C]DA (Table 2) above the basal levels in the presence of RES as well as of TBZ and, concomitantly, significant stimulations of [^{14}C]DA synthesis may occur (Fig. 2). Therefore, TBZ, like RES, does appear to elevate the level of AMT releasable cytoplasmic amine, and thus intraneuronal actions of TBZ seem indistinguishable from those of RES. However, PCP (Fig. 1) stimulated labeled DA formation at the lower medium TBZ levels in contrast to the effects seen in the presence of RES. Only at 1.80 μM TBZ was any significant PCP inhibition of labeled DA formation seen. AA also enhanced [^{14}C]DA formation at various TBZ (Fig. 3) levels (except 1.80 μM) but inhibited at any RES concentration. The present results, therefore, indicate differential interactions of PCP (and also AA), but not of AMT, with TBZ and RES affecting synaptosomal DA.

It may be mentioned here that, for the results reported, the concentrations of the stimulants, PCP (9.1 μM), AMT (9.1 μM) and AA (0.91 μM), were selected on the basis of their comparable stimulations of [^{14}C]DA formation in the absence of TBZ or RES (Figs. 1–3). Furthermore, in the presence of RES these concentrations of the stimulants produced clear effects of either stimulation (AMT) or inhibition (PCP and AA) of [^{14}C]DA formation [17, 22]. Additionally, other concentrations of these drugs were also tested, and the results obtained were very similar to those reported. AMT at a 0.91 μM level increased labeled DA formation by 76.2 and 16.3% above the corresponding basal levels of 0.09 and 1.80 μM TBZ and by 27.3% above that of 1.80 μM RES. The stimulating effect of PCP in the presence of a low TBZ concentration (0.09 μM) was also confirmed (22.7% above the basal value) at the 3.1 μM level.

It is interesting to note that, as the concentration of TBZ was raised to 1.80 μM, the effects of PCP and AA on [^{14}C]DA formation were reversed from stimulation to inhibition. Also tested for confirmation were 0.91, 3.1 and 36.4 μM PCP with 1.80 μM medium TBZ and, again, inhibitions of 14.5, 32.6 and 24.5%, respectively, were observed. It was not consideded suitable to use a level of TBZ any higher than 1.80 μM because the resultant near total inhibition of [^{14}C]DA formation would make any observed PCP effect less reliable. If, indeed, these results (Fig. 1) at the 1.80 μM TBZ level suggest any specific action of PCP, it is noteworthy that a similar degree of [^{14}C]DA formation inhibition may be seen at a much lower concentration (0.022 μM) of RES. It is interesting that the appearance of the inhibitory effect at a relatively high concentration of TBZ may correlate not as much with the similar potencies of these two agents in depleting endogenous DA [21], releasing [^{14}C]DA or inhibiting [^{14}C]DA formation (Table 1) but rather with their widely different animal sedative potencies [19]; clinically, too, a considerably higher dose of TBZ (25–200 mg) compared to that of RES (1–9 mg) is required for sedative actions [32]. Thus, there may be a PCP and AA sensitive intraneuronal site which also has a characteristic of unequal affinities for TBZ and RES. However, for the storage sites contributing to the AMT releasable pool, these two releasing agents appear the have comparable affinities.

In summary, the present results indicate that the observed inhibitory actions of PCP and AA on DA formation in the presence of RES are probably not directly due to the broad DA releasing effects of the latter drug. It is possible, however, that the nonamphetamine stimulant-induced inhibitions are associated with rather a small amine pool that has been related to RES-induced depression [33], and that this pool may respond only to a relatively much higher level of TBZ. It is also possible that any DA release may be, as suggested before [34], an epiphenomenon of a more basic RES action at some site with an affinity for TBZ [19] that is relatively weak in influencing the nonamphetamine actions.

If, indeed, RES is found to be an effective antidote for PCP abuse syndrome as suggested before [17], the present results provide some indication that TBZ could also be useful for that purpose.

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