Monoamine Oxidase Inhibitors Antagonize the Acceleration of Brain Dopamine Synthesis Induced by Neuroleptic Drugs in vivo: Implications for the Treatment of Tardive Dyskinesia

HORNYKIEWICZ[1] has characterized neuroleptic (antipsychotic) drugs as agents which elicit a 'striatal dopamine deficiency syndrome' by blocking dopamine (DA) receptors in corpus striatum and limbic system (trifluoperazine) or by depleting neuronal stores of DA (tetrabenazine). Both types of neuroleptics induce a compensatory acceleration of DA synthesis[2]. This increase in synthesis rate is shown below to be very sensitive in vivo to monoamine oxidase (MAO) inhibitors, which can completely block the compensatory response.

The rate of DA synthesis was ascertained by measuring the accumulation of dopa[2–4] in rat corpus striatum 30 min after the administration of 0.75 mmole/kg i.p. of the aromatic L-amino acid decarboxylase inhibitor, NSD-1024 (3-hydroxy-*O*-benzylhydroxylamine hydrochloride)[5]. Sprague-Dawley male rats (Charles River Breeding Laboratories, Wilmington, Mass.), 180–230 g, in groups of 5 received the various pretreatments indicated in Figures 1–3 before NSD-1024. Dopa and DA were isolated from corpus striatum via AG50WX4 Na columns[2,6], purified on acid-washed Woelm alumina, and determined in the final 0.1 *N* HCl eluates by the iodine oxidation method[7] in a Technicon AutoAnalyzer[8]. Monoamine oxidase activity was assayed by the method of WURTMAN and AXELROD[9]. All drugs were hydrochlorides except tetrabenazine.

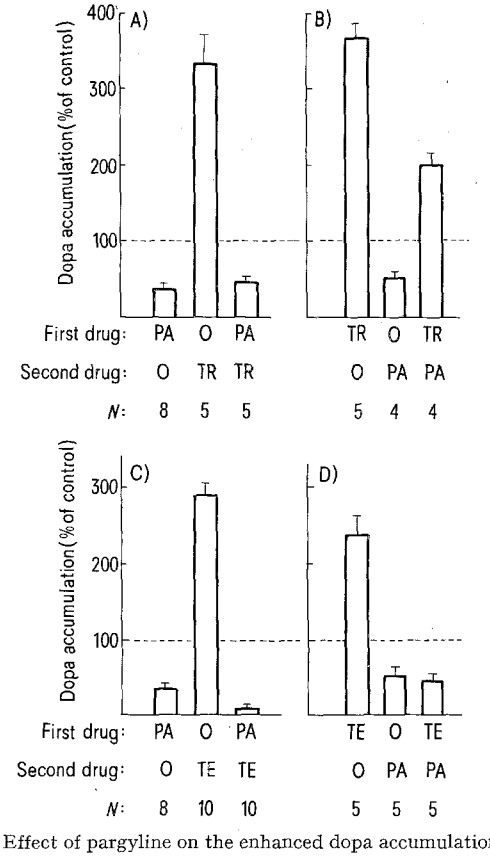

Fig. 1. Effect of pargyline on the enhanced dopa accumulation in rat corpus striatum induced by trifluoperazine or tetrabenazine. Rats in groups of 5 received the 2 i.p. treatments indicated in the figure, 30 min apart, followed by NSD-1024, 0.75 mmole/kg (132 mg/kg) i.p., 30 min after the second drug. Rats were sacrificed 30 min following administration of the decarboxylase inhibitor. Doses: pargyline (PA), 178 μmole/kg (34.8 mg/kg); trifluoperazine (TR), 4 μmole/kg (1.7 mg/kg); tetrabenazine (TE), 4 μmole/kg (1.3 mg/kg); water (0), 5 ml/kg. Bars represent means ± S.E. Corpus striatum of rats receiving only NSD-1024 (controls) contained 1.004 ± 0.057 μg/g dopa ($N = 13$). The dopa accumulation of all treatments differed from that of controls ($p < 0.01$ to < 0.001). In addition, in 1A: $p < 0.001$ for PA + O vs. O + TR and O + TR vs. PA + TR; in 1B and 1C: the 3 treatments in each group differed from each other ($p < 0.001$); in 1D: $p < 0.001$ for TE + O vs. O + PA and TE + O vs. TE + PA. *N* is number of rats.

Administration of 4 μmole/kg i.p. of trifluoperazine or tetrabenazine followed by the decarboxylase inhibitor elicited dopa accumulations 3 to 4 times that found in control rats receiving NSD-1024. These data agree with the acceleration of DA synthesis ascertained by measuring the rate of formation of DA from ^{14}C-tyrosine[10] or determining the increase in tyrosine hydroxylase activity of brain preparations[11]. Pargyline (MAO inhibitor) itself reduced dopa accumulation, in agreement with the deceleration of DA synthesis reported previously[12,13]. Combining pargyline with trifluoperazine gave less dopa accumulation than that shown by control rats (Figure 1A), whereas pargyline + tetrabenazine virtually halted dopa formation (Figure 1C). Reversing the pretreatments to neuroleptic + pargyline still resulted in a pronounced decrease in the enhanced dopa accumulation normally obtained (Figures 1B and 1D). Antagonism of the accelerated synthesis of DA elicited by the neuroleptic drugs increased with the pargyline dose (Figures 1A, 1C, and 2). The MAO inhibitor pheniprazine also blocked the acceleration of DA synthesis (Figure 3); when combined with tetrabenazine, dopa was hardly formed.

The increase in DA levels after the MAO inhibitors was consistent with the decrease in MAO activity of brain homogenates (Table). The decrease in DA content was pronounced after tetrabenazine and slight after trifluoperazine (Table)[2]. The elevated concentration of DA elicited by the MAO inhibitors is probably responsible for the marked antagonism of the accelerated DA synthesis induced by neuroleptic drugs, because DA itself inhibits tyrosine hydroxylase[12,13]. The effect on tetrabenazine is greater than that on trifluoperazine (Figures 1–3). More free DA is probably available after MAO inhibitor + tetrabenazine, thereby resulting in greater inhibition of tyrosine hydroxylase and cut back in dopamine synthesis.

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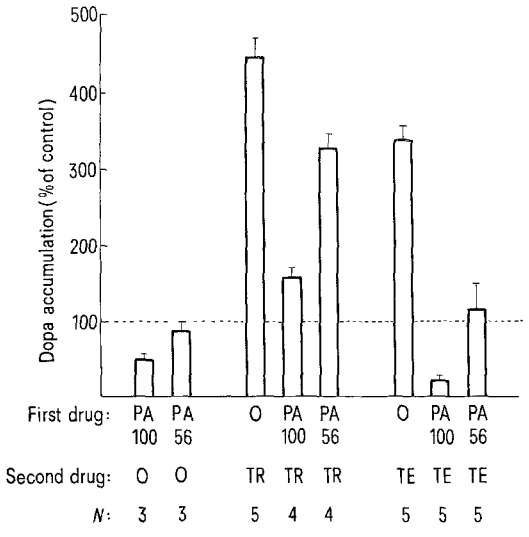

Fig. 2. Effect of pargyline on the enhanced dopa accumulation in rat corpus striatum induced by trifluoperazine or tetrabenazine. Drugs were administered as described in Figure 1. Doses: pargyline (PA), 100 μmole/kg (19.6 mg/kg) and 56 μmole/kg (11.0 mg/kg); trifluoperazine (TR), 4 μmole/kg (1.7 mg/kg); tetrabenazine (TE), 4 μmole/kg (1.3 mg/kg); water (O), 5 ml/kg. Bars represent means $\pm$ S.E. Corpus striatum of rats receiving only NSD-1024 (controls) contained 0.784 $\pm$ 0.070 μg/g dopa ($N = 5$). The dopa accumulation of all treatments differed from that of controls ($p < 0.02$ to < 0.001), except for PA (56) + O and PA (56) + TE. In the TR and TE groups the 3 treatments in each group differed from each other ($p < 0.05$ to $p < 0.001$).

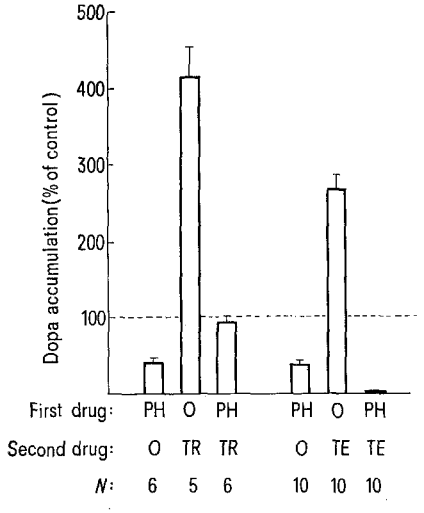

Fig. 3. Effect of pheniprazine on the enhanced dopa accumulation in rat corpus striatum induced by trifluoperazine or tetrabenazine. Drugs were administered as described in Figure 1. Doses: pheniprazine (PH), 32 μmole/kg (6.0 mg/kg); trifluoperazine (TR), 4 μmole/kg (1.7 mg/kg); tetrabenazine (TE), 4 μmole/kg (1.3 mg/kg); water (O), 5 ml/kg. Bars represent means $\pm$ S.E. Corpus striatum of rats receiving only NSD-1024 (controls) contained 0.851 $\pm$ 0.042 μg/g dopa ($N = 15$). The dopa accumulation of all treatments differed from that of controls ($p < 0.001$), except for PH + TR. In the TR and TE groups the 3 treatments in each group differed from each other ($p < 0.01$ to < 0.001).

Tardive dyskinesia, a neurological disorder which may appear on longterm neuroleptic therapy, is considered to be caused by the supersensitization of DA receptors induced on prolonged blockade with antipsychotic drugs[14]. Treatment[15] has consisted of counteracting this hypersensitivity with tetrabenazine (to deplete DA) or haloperidol (to block receptors)[16]. The potential of a 'vicious circle' in this situation has been pointed out[15], since etiology and therapy both involve neuroleptic drugs. Marked improvement of dyskinesia has been observed with administration of a MAO inhibitor (isocarboxazide) along with chlorpromazine[17]. Further study of the antidyskinetic action of MAO inhibitors has been recommended, although the beneficial effect has not been satisfactorily explained[15]. My results suggest that the efficacy of MAO inhibition in tardive dyskinesia may be derived from the suppression of the increased DA synthesis associated with antipsychotic drugs. Enhancement of chlorpromazine's antipsychotic activity and prevention of its extrapyramidal effects by the coadministration of MAO inhibitors (pargyline, pheniprazine, tranylcypromine) have also been reported[18]. Also pertinent is the potentiation of the therapeutic effects of thioridazine and chlorpromazine in chronic schizophrenic patients by the tyrosine hydroxylase inhibitor, α-methyltyrosine[19], which would be expected to block the accelerated synthesis of DA elicited by the two phenothiazines.

Dopamine is metabolized by types A and B MAO enzymes, whereas serotonin and norepinephrine are preferred substrates of type A MAO[20]. Since selective inhibition of type B MAO presumably elevates only DA levels in brain[20], one might speculate that DA-specific

Effect of drugs on dopamine content of rat corpus striatum and monoamine oxidase activity of whole rat brain homogenates

Treatment Drug	Dose i.p.		DA content of corpus striatum	MAO activity of whole brain
	μmole /kg	(mg/kg)	(% control)	(% inhibition)
Pargyline	178	(34.8)	121 $\pm$ 7 [a]	95 $\pm$ 0 [d]
	100	(19.6)	117 $\pm$ 5 [b]	61 $\pm$ 1 [d]
	56	(11.0)	108 $\pm$ 3	26 $\pm$ 2 [d]
Pheniprazine	32	(6.0)	132 $\pm$ 8 [c]	90 $\pm$ 1 [d]
Trifluoperazine	4	(1.7)	85 $\pm$ 5 [a]	—
Tetrabenazine	4	(1.3)	13 $\pm$ 1 [d]	—

Rats received drugs or water (control) 60 min before sacrifice. Data entries are means $\pm$ S.E. (5 rats per treatment). Corpus striatum of control rats contained 10.99 $\pm$ 0.33 μg/g dopamine. After similar treatments with pargyline, pheniprazine, or water (control) the MAO activity of each whole brain homogenate was determined in duplicate. Data entries are means $\pm$ S.E. (3 rats per treatment). Differences from control mean values are indicated by p numbers. [a] $p < 0.05$. [b] $p < 0.02$. [c] $p < 0.01$. [d] $p < 0.001$.

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MAO inhibitors would be of therapeutic value for enhancing antipsychotic activity and attenuating or controlling side-effects which may originate from the excessive formation and turnover of DA induced by antipsychotic drugs.

Zusammenfassung. Pargylin und Pheniprazin vermindern stark die Dopa-Anreicherung (erhöhte Dopamin-Synthesegeschwindigkeit) im corpus striatum der Ratte nach Trifluoperazin und Tetrabenazin. Dopamin-spezifische MAO-Hemmer könnten deshalb therapeutischen Wert haben in der Kontrolle von tardiver Dyskinesia, die möglicherweise durch erhöhte Bildung und Umsatz von Dopamin unter dem Einfluss von anti-psychotischen Drogen hervorgerufen wird.

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Increased Vascular Permeability Induced in Synovialis of the Rat by Histamine, Serotonin and Bradykinin

Histamine, serotonin and bradykinin evoke increased vascular permeability when injected into some but not all mammalian tissues. In the rat, histamine induces a permeability response in skin, subcutaneous tissue, skeletal muscle, pleura and peritoneum, but not in testis, kidney or brain[1]. No studies of the effect of histamine, serotonin or bradykinin on the vascular permeability of synovialis of the rat have been reported, although histamine is known to increase the vascular permeability of synovialis in the monkey[2] and rabbit[3]. Further, the relative increase in vascular permeability induced by the above substances has been studied mainly in the skin of various laboratory animals[4] and little is known of their corresponding effects in other tissues.

This paper reports an investigation of the relative effects of histamine, serotonin and bradykinin on the vascular permeability of the synovial membrane in the stifle joint of the rat, and an electron microscopic study of synovial vessels rendered abnormally permeable by these substances.

Materials and methods. Albino rats of both sexes (body weight 250–350 g) were used, being lightly anaesthetized with ether for all injections. Serial 10-fold dilutions of histamine acid phosphate (0.36–360 µg histamine base/ml), serotonin creatinine sulphate (0.005–50 µg serotonin base/ml) and bradykinin (0.01–10 µg/ml) were prepared in Tyrode solution, pH 7.3; 0.05 ml of each dilution of each substance was injected into both the left and right stifle joints of 5 rats. The stifle joints of a further 5 rats, injected with 0.05 ml Tyrode solution alone, served as controls.

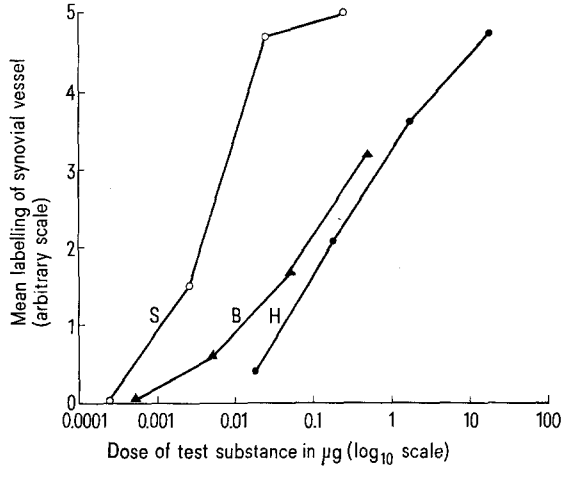

Fig. 1. Increased vascular permeability (assessed by carbon labelling of synovial vessels) induced in rat's synovialis by serotonin (S), bradykinin (B) and histamine (H). The mean score of the control-stratum synoviale was 0.25.

Increased vascular permeability induced in the synovialis was detected by injecting each animal i.v. with colloidal carbon (Gunther Wagner, C11/1431a, Pelikan, Hanover), 0.1 ml/100 g body weight, just prior to injection of the joints with the above substances. Circulating colloidal carbon is removed from the blood by the reticulo-endothelial system within 1 h, but during that period, carbon also collects in the walls of abnormally permeable blood vessels[5]. 75 min after the i.v. injection of colloidal carbon, each animal was killed and the stifle joints were opened to expose the synovialis. The synovial membranes were fixed in formaldehyde, 'cleared' in glycerol and examined with a dissecting microscope. The amount of carbon deposited in the walls of the synovial vessels (referred to as 'labelling' of the vessels) of each joint served as an index of the increased vascular permeability and was scored on an arbitrary scale 0 to 5. 16 of the 150 joints in the series contained blood at dissection and were excluded from the results as haemorrhage into the joint cavity is known to increase the permeability of the synovial microvasculature[6].

Synovial membranes were prepared for electron microscopy following intra-articular injection of 1.8 µg histamine base, 0.25 µg serotonin base and 0.5 µg bradykinin, respectively. The animals were killed 8 min after giving i.v. colloidal carbon and injection of the joints with each substance: the synovial membranes were removed and fixed in combined aldehyde fixative[7] and osmium tetroxide. Epon embedded sections were stained with uranyl acetate and lead citrate, and examined with a Philips 300 electron microscope.

Results and discussion. Histamine, serotonin and bradykinin each increased the vascular permeability of synovialis in the range of doses tested (Figure 1). Histamine had least potency, the minimum dose required to induce labelling of synovial vessels being 0.018 µg per joint. Maximal labelling of synovial vessels was obtained with a dose of 18 µg histamine per joint. Serotonin had

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