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High affinity binding of [^{3}H]-tyramine in the central nervous system

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1 Optimum assay conditions for the association of [^{3}H]-*para*-tyramine ([^{3}H]-pTA) with rat brain membranes were characterized, and a saturable, reversible, drug-specific, and high affinity binding mechanism for this trace amine was revealed.

2 The binding capacity (B_{max}) for [^{3}H]-pTA in the corpus striatum was approximately 30 times higher than that in the cerebellum, with similar dissociation constants (K_D).

3 The binding process of [^{3}H]-pTA involved the dopamine system, inasmuch as (a) highest binding capacity was associated with dopamine-rich regions; (b) dopamine and pTA equally displaced specifically bound [^{3}H]-pTA; (c) there was a severe loss in striatal binding capacity for [^{3}H]-pTA and, reportedly, for [^{3}H]-dopamine, following unilateral nigrostriatal lesion; (d) acute *in vivo* reserpine treatment markedly decreased the density of [^{3}H]-pTA and, reportedly, of [^{3}H]-dopamine binding sites.

4 In competition experiments [^{3}H]-pTA binding sites, though displaying nanomolar affinity for dopamine, revealed micromolar affinities for the dopamine agonists apomorphine and pergolide, and for several dopamine antagonists, while having very high affinity for reserpine, a marker for the catecholamine transporter in synaptic vesicles.

5 The binding process of [^{3}H]-pTA was both energy-dependent (ouabain-sensitive), and ATP-Mg^{2+}-insensitive; furthermore, the potencies of various drugs in competing for [^{3}H]-pTA binding to rat striatal membranes correlated well ($r = 0.96$) with their reported potencies in inhibiting [^{3}H]-dopamine uptake into striatal synaptosomes.

6 It is concluded that [^{3}H]-pTA binds at a site located on/within synaptic vesicles where it is involved in the transport mechanism of dopamine.

Introduction

Increasing interest is being focused on the functional role of 'trace amines' in neural transmission. Trace amines are those biogenic compounds such as tyramine, phenylethylamine, phenylethanolamine, octopamine, synephrine, and tryptamine which are normally present in nanomolar concentrations per g of neural tissue (Boulton, 1976). The *para*- and *meta*-isomers of tyramine (pTA, mTA) are highly concentrated in the rat striatum, where they appear to be stored by a reserpine-sensitive mechanism (Boulton *et al.*, 1977). Inasmuch as their concentrations are impaired by administration of dopaminergic antipsychotics or by drugs that interfere with amine storage, and increased by drugs that reduce dopamine turnover (see Juorio, 1982, for references), striatal tyramines are thought to act as modulators of the activity of dopaminergic neurones (Juorio, 1979). It is, therefore, conceivable that alterations in pTA or other trace amine homeostasis following, or coinciding with a diseased dopaminergic system, are associated with the origin of several mental disorders, principally depression (Boulton, 1984, Sandler *et al.*, 1984).

Using electrophysiological techniques it has recently been shown that some of the neuronal effects of trace amines might be related to actions on specific 'receptors' and, as such, they may possess a transmitter role (Jones, 1984). The possible existence of specific receptors for pTA in the brain has been further postulated on the basis of the increase in motor activity provoked by an intraventricular injection of pTA to rats, pretreated with reserpine or α-methyl-*p*-tyrosine in order to exclude the possibility of indirect receptor stimulation (Stoof *et al.*, 1976).

The purpose of the present study was to characterize the association of [^{3}H]-pTA with membranes isolated from the rat brain. It was shown that pTA has high

affinity for a dopaminergic site, most probably involved in the vesicular transport mechanism of dopamine.

A preliminary account of this work has been published (Vaccari, 1985).

Methods

Preparation of tissues

Male Sprague-Dawley (Nossan, Milano) rats (250–300 g) were used. All dissections of the brain were performed on ice, and soon after tissues were processed for homogenization.

The cerebral cortex included the hippocampus, amygdala and septum. The corpus striatum was separated from the neocortex at the radiation of the corpus callosum, and from the diencephalon at the sulcus terminalis. Dissection of the entire hypothalamus was limited anteriorly by the margin of the optic chiasma, laterally by the lateral fissures, and posteriorly by the margin of the mammillary body. The block was about 2.5 mm deep from the basal surface of the hypothalamus. The pons-medulla was separated from the mesodiencephalon by an oblique cut running from the posterior border of the inferior colliculus on the dorsal surface, to the anterior border of the pons on the ventral surface. The pons-medulla was separated from the spinal cord by cutting below the obex.

Tissues were manually disrupted, and then homogenized (800 r.p.m., 10 strokes up-down, glass-glass homogenizers) on ice in 10 ml of 50 mM Tris-HCl buffer (pH 7.02 at 37°C) containing 120 mM NaCl and 5 mM KCl. Homogenates were then diluted up to a final volume of 40 ml, sonicated for 15 s, and centrifuged thrice at 48,000 *g* for 10 min. The pellets obtained after the first centrifugation were vortex-suspended in 20 ml buffer, incubated at 37°C for 10 min in order to inactivate endogenous ligands, and then further diluted to 40 ml. The final pellets were manually homogenized (1:40 w/v) with ice-cold buffer (incubation buffer) supplemented with 10 μM pargyline and 50 μM ascorbic acid, a concentration which did not influence the binding reaction of [^{3}H]-pTA.

[^{3}H]-para-tyramine binding

Optimum conditions for [^{3}H]-pTA binding were as follows. Soon after preparation, membrane aliquots of the 1:40 (w/v) suspension (100 μl, approx 2.2 mg protein ml^{-1}) were incubated with [^{3}H]-pTA (0.5–32 nM) for 10 min at 37°C in the absence or presence of 10 μM pTA (as the displacing agent) in a final volume of 1 ml. Samples were then replaced on ice, rapidly (3–4 s) filtered through Whatman GF/B filters, and washed with 3 × 4 ml ice-cold incubation buffer. Filters were allowed to stand overnight at room temperature in 5 ml Aquasol-2 (New England Nuclear) before being counted. Specific binding of 4 nM [^{3}H]-pTA to striatal membranes amounted to approx. 90% of total binding; binding to the filters was minimal.

Neuronal lesions

The animals were anaesthetized with ketamine (Ketalar, Parke Davis, 100 mg kg^{-1} i.p.) and placed in a stereotaxic apparatus. Unilateral lesioning of nigrostriatal fibres was performed (Toffano *et al.*, 1983) by inserting a 4 mm wide stainless steel blade next to the midline (1 mm posterior to bregma, 0.5 mm lateral to the midline, and 9 mm deep, at an angle of 68°). The success of the lesion was inferred from the residual synaptosomal uptake of [^{3}H]-dopamine (10% of that in the striatum of the controlateral unlesioned side) measured in the lesioned side (Raiteri *et al.*, 1985). Residual [^{3}H]-dopamine uptake was considered as an index of dopamine-nerve terminals surviving the lesion.

Protein samples

Protein concentrations were assayed by a modification of the method of Lowry (Peterson, 1977) using bovine serum albumine as the standard.

Calculations

Eadie-Hofstee plots for saturation curves, and binding parameters were calculated according to Zivin and Waud (1982), with correction for bias inherent to this analysis. The IC_{50} values (the concentration of each compound that inhibited by 50% the specific binding of 4 nM [^{3}H]-pTA in competition experiments) were calculated graphically, and then transformed to K_i values according to the Cheng-Prusoff equation (Cheng & Prusoff, 1973):

$$K_i = \frac{IC_{50}}{1 + \frac{nM}{K_D}}$$

where nM = the concentration of [^{3}H]-pTA used, and K_D = the dissociation constant (nM) of the ligand. Results were analysed by standard or paired Student's *t* tests.

Drugs

The drugs used were obtained from the following sources either commercially or, when marked*, as generous gifts which are gratefully acknowledged. *p*-

Tyramine [ring-^{3}H]·HCl (29–35 Ci mmol^{-1}) (New England Nuclear); *m*-tyramine (Vega Biotechnologies, Dr G. Baker*, Edmonton, Canada); domperidone, haloperidol, ketanserin, and spiperone (Janssen Pharmaceutica*), dibenamine, furmethide, propranolol (Smith Kline & French Laboratories*); *cis*(Z)-flupenthixol (Lundbeck & Co.*), and citalopram (Lundbeck & Co.*, courtesy of Prof. M. Raiteri, Genova); D-butaclamol (Ayerst Research Labs.*); (±)-sulpiride (Ravizza*); nomifensine (Hoechst*); pergolide (Lilly Research Labs.*, kindly supplied by Prof. M. Raiteri); DL-methyl-amphetamine (Zilliken*); methylphenidate (Ciba*); apomorphine, benztropine, chlorpromazine, 2,4-dinitrophenol, L-DOPA, dopamine, (−)-adrenaline, 5-hydroxytryptamine, imipramine, (−)-noradrenaline, (±)-octopamine, ouabain, (−)-phenylephrine, β-phenylethylamine, reserpine, saponin, *p*-tyramine, L-tyrosine, adenosine 5′-triphosphate (ATP), guanosine 5′-monophosphate (GMP), guanosine 5′-diphosphate (GDP), and guanosine 5′-triphosphate (GTP) were obtained from Sigma; atropine and nicotine (B.D.H.); D-amphetamine (Gianni); hydroxymercuribenzoic acid and N-ethylmaleimide (Serva Feinbiochemia); dithiothreitol (Calbiochem). All drugs were dissolved immediately before use in buffer, 0.01 N HCl, or ethanol, then further diluted with water, buffer, or 0.01 N HCl. The original isotope was diluted 1:4 (v/v) with 0.01 N HCl,and working dilutions were made up with incubation buffer.

Results

Factors influencing the binding of [^{3}H]-para-*tyramine*

Protein linearity Using whole brain (less cerebellum) membranes, the specific binding of [^{3}H]-pTA (4 nM) was linear in the range 0.02–0.3 mg protein per ml of incubation volume, after which binding saturated. All assays were routinely performed with 0.2–0.25 mg protein.

pH- and temperature-dependence [^{3}H]-pTA binding was pH- and temperature-dependent. Using Tris-HCl buffer, the maximum specific binding was observed at pH 6.7, with a gradual decrease occurring at more basic pH values, so that binding at pH 7.0, 7.5, and 8.0 was 78%, 36%, and 4% of that at pH 6.7, respectively. Binding assays were routinely performed at pH 7.0.

Association and dissociation The rate of association of [^{3}H]-pTA was very low at 4°C, the equilibrium not being attained even 120 min after the start of incubation. At 37°C, the temperature chosen for standard assays, half-maximum association values were reached at approx. 4 min, and equilibrium was attained by 10 min, afterwards there was a rapid decline in the amount of ligand specifically bound to striatal membranes. After 30 min of incubation, the specific binding of [^{3}H]-pTA had almost totally disappeared. A 6 fold dilution with warm (37°C) incubation buffer at the end of the standard binding reaction, and subsequent filtration of samples at various times following dilution, provoked a rapid and complete dissociation of the specifically bound ligand, with a $t_{\frac{1}{2}}$ of less than 3 min.

Sodium-dependence The binding of [^{3}H]-pTA was completely dependent upon the presence of Na^+ ions in the incubation medium. [^{3}H]-pTA did not bind to whole brain membranes in the absence of NaCl; also specific binding with 60 mM NaCl was approx 38% of that at 120 and 240 mM NaCl.

Nucleoside phosphates The binding reaction of [^{3}H]-pTA (8 nM) was not affected by ATP (up to 2 mM) or ATP plus 1 mM $MgSO_4$ in the incubation medium, but it was inhibited (by 25%) by the addition of 1 mM $MgSO_4$ (Table 1).

The guanyl nucleotides GTP, GDP and GMP consistently (by 49%, 49%, and 24%, respectively)

Table 1 Effects of nucleoside phosphates, inhibitors of energy metabolism, and membrane reagents on the specific binding of [^{3}H]-*para*-tyramine ([^{3}H]-pTA) to rat striatal membranes

Addition	*Specifically bound* (% of controls)	n
ATP 2×10^{-4} M	76.8 ± 13.3	8
ATP 2×10^{-3} M	82.9 ± 14.7	8
GTP 1×10^{-3} M	51.0 ± 11.4***	7
GDP 1×10^{-3} M	50.5 ± 6.4***	7
GMP 1×10^{-3} M	75.5 ± 7.3**	7
$MgSO_4$ 1×10^{-3} M	74.9 ± 9.8*	5
$MgSO_4$ 1×10^{-3} M + ATP 2×10^{-3} M	86.5 ± 13.8	4
Ouabain 1×10^{-4} M	14.0 ± 4.1***	5
Ouabain 1×10^{-4} M + ATP 2×10^{-3} M	12.1 ± 4.9**	5
Dinitrophenol 1×10^{-4} M	0.6 ± 0.4**	5
Saponin 0.2%†	3.4 ± 2.2**	4
N-ethylmaleimide 1×10^{-4} M	9.3 ± 0.8	2
Hydroxymercuribenzoic acid 1×10^{-4} M	13.5 ± 0.9	2
Dithiothreitol 1×10^{-4} M	94.2 ± 8.3	2

The binding reaction of [^{3}H]-pTA (8 nM) was performed in the absence or presence of drugs. † Saponin 0.2% (final concentration) was added at the end of the binding reaction, thereafter samples were incubated on ice for 10 min. Values are mean ± s.e.mean of *n* experiments performed in triplicate. *$P < 0.05$; **$P < 0.02$ or < 0.01; ***$P < 0.005$ or < 0.001, versus respective controls (paired *t* test).

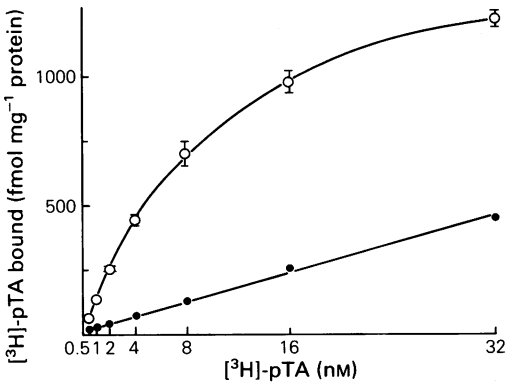

Figure 1 Saturation of specifically bound [^{3}H]-*para*-tyramine ([^{3}H]-pTA) (0.5–32 nM) (○) to rat striatal membranes, and non-specific binding (●) as measured with 10 μM pTA as the displacer. Results are means, with vertical lines showing s.e.means, from $n = 10$ experiments performed in triplicate.

Table 2 Brain and tissue distribution of specific [^{3}H]-*para*-tyramine ([^{3}H]-pTA) binding in the rat

Region	B_{max}	K_D	n
Corpus striatum	1658.7 ± 69.1	11.5 ± 0.8	10
Hypothalamus	228.6 ± 39.9	11.0 ± 1.3	5
Cortex	154.5 ± 33.7	11.5 ± 1.1	5
Pons-medulla	93.4 ± 13.9	8.0 ± 1.9	5
Cerebellum	54.0 ± 7.7	10.7 ± 2.8	4
Heart	not detectable		2
Kidney	not detectable		2
Liver	not detectable		2

B_{max} = fmol mg^{-1} protein; K_D = nM.
Values are means ± s.e.mean from n separate experiments each with 7 concentrations (0.5–32 nM) of [^{3}H]-pTA, determined in triplicate. Binding parameters were calculated with Eadie-Hofstee analysis, and corrected for bias (Zivin & Waud, 1982).

inhibited the specific binding of [^{3}H]-pTA, when added at the start of the binding reaction (Table 1).

Metabolic inhibitors Inhibitors of energy metabolism such as 100 μM ouabain or dinitrophenol potently inhibited the binding of [^{3}H]-pTA, and ouabain-induced inhibition was not affected by the addition of 2 mM ATP to the incubation medium (Table 1). When membranes were incubated with [^{3}H]-pTA (8 nM) under standard conditions, then returned to ice, and incubated again for 10 min on ice in the presence of 0.2% saponin in order to make the membranes permeable to any accumulated ligand, the specifically bound radioactivity almost totally disappeared (Table 1).

Table 3 Inhibition of specific [^{3}H]-*para*-tyramine ([^{3}H]-pTA) binding to rat striatal membranes by a variety of active compounds

	K_i (nM)	n
Dopamine-related agents		
Apomorphine	6825 ± 372	3
L-DOPA	1298	2
Dopamine	13.2 ± 2.6	6
Octopamine	292 ± 121	3
Pergolide	3858	2
β-Phenylethylamine	425 ± 91	3
p-Tyramine	16.6 ± 3.1	6
m-Tyramine	15.0 ± 3.5	5
L-Tyrosine	> 74,000	2
Neuroleptics		
(+)-Butaclamol	3189 ± 323	3
Chlorpromazine	3857	2
Domperidone	705 ± 130	3
cis(Z)-Flupenthixol	1311 ± 91	6
Haloperidol	556	2
Reserpine	5.5 ± 1.2	5
Spiperone	1075	2
(±)-Sulpiride	> 50,000	4
Monoamine uptake blockers		
D-Amphetamine	153 ± 9	3
Benztropine	90	2
Citalopram	36,700	2
Imipramine	13,724	2
Metyl-amphetamine	149 ± 31	3
Methylphenidate	144 ± 5	3
Nomifensine	95 ± 21	4
Adrenoceptor agents		
Dibenamine	3115	2
(−)-Adrenaline	430	2
(−)-Noradrenaline	92 ± 22	3
Phenylephrine	1743	2
Propranolol	22,255	2
Cholinoceptor agents		
Acetylcholine	> 60,000	2
Atropine	14,837	2
Furtrethonium	> 100,000	2
Nicotine	> 100,000	2
5-Hydroxytryptamine-receptor agents		
5-Hydroxytryptamine	668	2
Ketanserin	3500	2

The binding reaction of [^{3}H]-pTA (4 nM) was performed in the absence or presence of competing drugs. The IC_{50} values (the concentration of drug required to inhibit 50% of the specifically bound [^{3}H]-pTA) were transformed to K_i values by using a K_D value of 11.5 nM in the Cheng-Prusoff equation (see Methods). Values are means ± s.e.mean of n experiments performed in duplicate over a range of 10 concentrations of each drug.

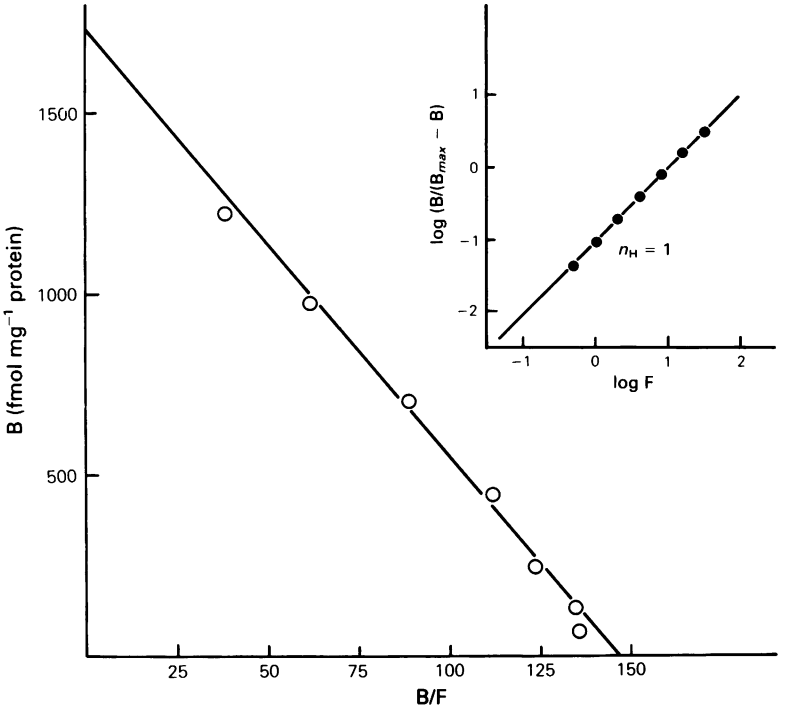

Figure 2 Eadie-Hofstee and Hill (inset) plots for the binding curve of [^{3}H]-*para*-tyramine ([^{3}H]-pTA) shown in Figure 1. B = specifically bound [^{3}H]-pTA; F = free [^{3}H]-pTA (0.5–32 nM); B_{max} = 1659 fmol mg^{-1} protein. Lines were fitted by linear regression analysis with the least squares method. The Hill constant (slope), n_H = 1.0.

Thiol reagents Alkylation of protein sulphydryl (-SH) groups with high concentrations of N-ethylmaleimide or hydroxymercuribenzoic acid inhibited the binding process of [^{3}H]-pTA to striatal membranes (Table 1). Conversely, the reduction of protein disulphide (-S-S-) bonds by dithiothreitol did not affect [^{3}H]-pTA binding sites (Table 1).

*Saturation of [^{3}H]-*para*-tyramine binding*

The specific binding of [^{3}H]-pTA was saturable, whereas the non-specific binding increased linearly in the concentration range 0.5–32 nM (Figure 1). In the range 4–16 nM, [^{3}H]-pTA specifically bound to striatal membranes was approx. 5 fold as high as the non-specific binding. Eadie-Hofstee analysis (Figure 2) gave a straight line indicating a single population of non-interactive binding sites. The mean equilibrium dissociation constant (K_D) for striatal membranes was 11.5 ± 0.8 nM (n = 10), with a mean maximum binding (B_{max}) of 1659 ± 69 fmol mg^{-1} protein (n = 10). Hill analysis (Figure 2) showed no co-operativity, with a Hill number n_H = 1.

Brain regional, and tissue distribution of binding

The specific binding of [^{3}H]-pTA was unevenly distributed in the brain (Table 2), with the highest concentrations of binding sites being in the corpus striatum and, to a lesser extent, in the hypothalamus. The poorest region was the cerebellum, with a B_{max} value approx. thirty times smaller than that in the striatum. No specific binding of [^{3}H]-pTA could be measured in membranes from heart, kidneys and liver (Table 2).

*Drug specificity of [^{3}H]*para*-tyramine binding*

A variety of drugs were tested for their ability to compete for the binding of [^{3}H]-pTA (4 nM) to rat striatal membranes (Table 3). Dopamine potently displaced [^{3}H]-pTA, with the same K_i values as the tyramine isomers (in the range 13–16 nM). Dopamine-related agonists such as apomorphine and pergolide, the dopamine precursors tyrosine and DOPA and the tyramine derivative octopamine, were fairly weak or very poor displacers of [^{3}H]-pTA binding, with K_i

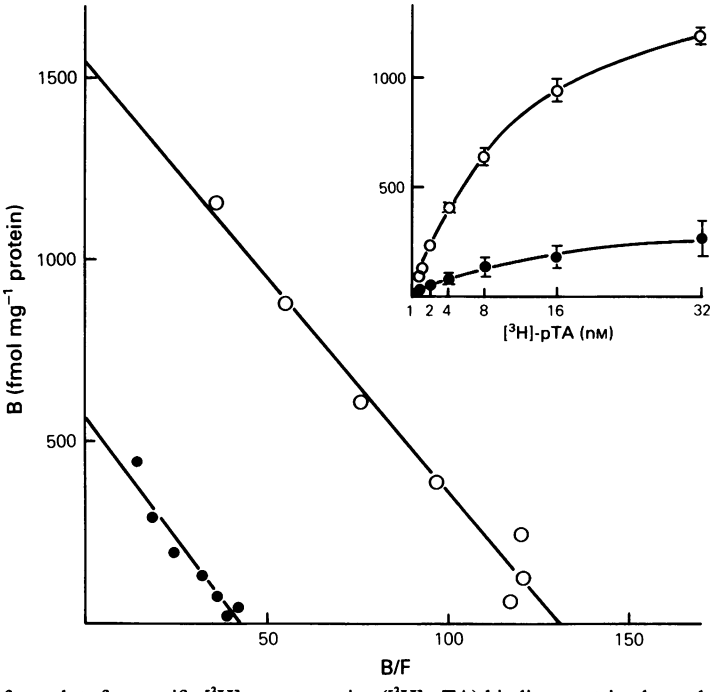

Figure 3 Eadie Hofstee plots for specific [^{3}H]-*para*-tyramine ([^{3}H]-pTA) binding to striatal membranes 8 days after unilateral, surgical lesion of the nigro-striatal dopamine pathway (●), and in the controlateral, non-lesioned striatum (○). The inset shows corresponding saturation curves. Results are means, with vertical lines showing s.e.mean, from $n = 6$ experiments performed in triplicate (saturation curves and controls in Eadie-Hofstee plots), or from $n = 4$ experiments (Eadie-Hofstee plots for lesioned striata). For additional details see legend of Table 4.

Table 4 Effects of nigro-striatal hemitransection on the specific binding of [^{3}H]-*para*-tyramine ([^{3}H]-pTA) to striatal membranes

Time after lesion	*[^{3}H]-pTA binding*	*Non-lesioned side*	*Lesioned side*	*% variation*	n
8 days	B_{max}	1666 ± 112	343 ± 115***	−79	6†
	K_D	12.6 ± 1.1	—		
	B_{max}	1546 ± 125	515 ± 61**	−67	4
	K_D	12.0 ± 1.6	12.8 ± 1.5		
60 days	B_{max}	1824 ± 233	724 ± 341	−60	6‡
	K_D	12.7 ± 0.9	11.8 ± 2.1		
	B_{max}	1609 ± 207	1396 ± 293	−13	3
	K_D	12.7 ± 1.9	15.6 ± 1.7		
	B_{max}	2038 ± 427	53 ± 20*	−97	3
	K_D	12.6 ± 0.6	8.1 ± 2.2		

B_{max} = fmol mg^{-1} protein; K_D = nM. Mean values ± s.e.mean from n separate experiments each performed in triplicate.

† The specific binding of [^{3}H]-pTA in the lesioned side of 2 out of 6 rats was not detectable, compared to that in the controlateral striatum; binding parameters shown were therefore not obtained from all the rats used but from the 4 rats with measurable binding.

‡ [^{3}H]-pTA binding had almost completely recovered from the effects of the lesion in 3 out of 6 rats, but it was still severely depressed in the remaining 3 rats.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.005$ as compared to the respective controlateral, non-lesioned side; paired t test.

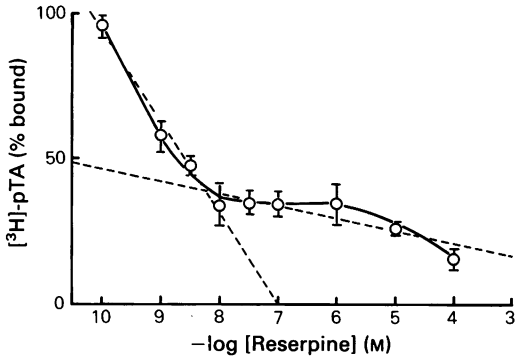

Figure 4 Reserpine inhibition of the binding of [^{3}H]-*para*-tyramine ([^{3}H]-pTA, 4 nM) to striatal membranes. Reserpine produced a two-phase competition curve and revealed high affinity ($K_i = 1.3 \pm 0.6$ nM) and low affinity ($K_i = 2.5 \pm 0.7$ μM) components of [^{3}H]-pTA binding. The straight lines for the two binding components were obtained by linear regression analysis on pooled data from 5 independent experiments. Vertical lines denote s.e.mean.

values in the range 0.3–70 μM. Butyrophenone neuroleptics, domperidone, haloperidol and spiperone required approx. micromolar concentrations to inhibit by 50% the specific binding of [^{3}H]-pTA. A similar finding was obtained with butaclamol, thioxantine (*cis*-Z-flupenthixol) and phenothiazine (chlorpromazine) antipsychotics. Sulpiride did not displace [^{3}H]-pTA binding, unless used at very large concentrations (Table 3).

The dopamine re-uptake blockers nomifensine, benztropine, D-amphetamine, methamphetamine and methylphenidate were fairly active displacers of [^{3}H]-pTA binding, at concentrations 10 to 15 fold higher than those of dopamine or the tyramines. Citalopram and imipramine, which block 5-hydroxytryptamine (5-HT) and noradrenaline uptake, respectively, did not affect [^{3}H]-pTA binding. The same was true for 5-HT receptor, cholinoceptor and adrenoceptor agents, the only exception being (−)-noradrenaline which had a K_i value of 91.5 nM; which is in the range of some dopamine re-uptake blockers.

Effects of a nigro-striatal hemitransection

The effects of surgical hemitransection of the nigro-striatal bundle, a procedure that decreases the uptake of [^{3}H]-dopamine (Raiteri *et al.*, 1985), was used as an index of damage to dopamine nerve terminals (Heikkila *et al.*, 1981) where endogenous pTA seems to be located (Juorio & Jones, 1981).

The number of [^{3}H]-pTA binding sites ipsilateral to the lesioned striatum 8 days following the lesion was severely (by 79%) decreased compared with the controlateral, non-lesioned side (Table 4 and Figure 3).

Since the specific binding of [^{3}H]-pTA was not detectable in the lesioned striatum of 2 out of 6 rats, binding parameters were calculated, based on the 4 samples with measurable binding (Table 4). Saturation curves were drawn using data from all the rats (Figure 3).

Two months following surgery, the specific binding of [^{3}H]-pTA to the lesioned side no longer differed from that to the controlateral side in 3 out of 6 rats (Table 4), thus indicating a recovery of the binding process. However, binding was still severely reduced in 3 rats (Table 4).

Nitro-striatal hemitransection consistently affected the B_{max}, whereas the affinity constant was similar in control and lesioned tissues.

In vivo *and* in vitro *effects of reserpine*

In order to ascertain whether the loss of [^{3}H]-pTA binding after nigro-striatal hemitransection was due to lesion-induced degeneration of dopamine nerve terminals, and/or to concomitant depletion of striatal dopamine stores, reserpine (Serpasil, Ciba), 5 mg kg^{-1} s.c., or saline was injected, and rats were killed 20 h post-injection. All reserpine-treated rats displayed the characteristic reserpine-induced symptoms (ptosis, hunch, diarrhoea), a loss of body weight in excess of 5% ($-9.1\% \pm 0.9$, $n = 6$), and the absence of post-decapitation convulsions, all reliable indicators of complete reserpinization (Halaris & Freedman, 1975). Reserpine administration consistently (by 86%) decreased the B_{max}, but did not affect K_D values of [^{3}H]-pTA binding to striatal membranes (Table 5).

[^{3}H]-pTA binding in the presence of increasing concentrations of reserpine was inhibited in a clearly biphasic

Table 5 Effects of reserpine on the specific binding of [^{3}H]-*para*-tyramine ([^{3}H]-pTA) to rat striatal membranes

Treatment	B_{max}	K_D
Saline	1753 ± 114	13.1 ± 0.9
Reserpine	252 ± 28***	14.1 ± 1.6

B_{max} = fmol mg^{-1} protein; K_D = nM. Mean values ± s.e.mean from $n = 6$ experiments performed in triplicate. Rats were injected s.c. with 5 mg kg^{-1} reserpine, or 0.9% NaCl (2 ml kg^{-1}), and killed 20 h later. ***$P < 0.001$ as compared to controls; standard t test.

manner, with a large plateau lying between approx. 10 nM and 1 μM reserpine (Figure 4). Thus, reserpine, allowed the definition of primary, high affinity (K_i = 1.3 nM), and secondary, low affinity (K_i = 2.5 μM) binding kinetics of [^{3}H]-pTA.

Discussion

A major finding of the present study is that [^{3}H]-pTA labels with high affinity (K_D approx. 10 nM) a site which is largely concentrated in membranes isolated from the corpus striatum and the hypothalamus, the two brain regions richest in endogenous dopamine. Regional distribution of [^{3}H]-pTA binding sites also strictly reflects the levels of endogenous tyramines in the brain (Philips, 1984); this correlation does not apply to the periphery, since no consistent binding of pTA could be detected in kidneys, heart and liver, despite their large content of tyramines (Philips, 1984).

The existence of an active accumulation process for tyramines in various nerve tissue preparations is well known (see Dyck, 1984, for references). Both pTA and mTA, and dopamine are taken up by rat striatal and hypothalamic slices by a high- and low-affinity process, and have similar kinetic values for the high-affinity component (Petrali *et al.*, 1979; Dyck, 1984). Furthermore, [^{3}H]-pTA is taken up by crude rat brain synaptosomes with a dissociation constant in the micromolar range (Ungar *et al.*, 1977), and by synaptic vesicles of the pig caudate nucleus (Lentzen & Philippu, 1977). However, the transport and binding processes of [^{3}H]-pTA seem to be separate entities, since: (a) lesions of the substantia nigra known to decrease the high affinity uptake of dopamine, did not affect the uptake of pTA in striatal slices (Petrali, 1977); (b) ATP-Mg^{2+}, which stimulates the uptake of [^{3}H]-pTA into synaptic vesicles (Lentzen & Philippu, 1977), did not affect the binding of [^{3}H]-pTA (Table 1); (c) the addition of reserpine to the standard incubation medium, in the absence of ATP-Mg^{2+}, markedly inhibited the binding of [^{3}H]-pTA (Figure 4), whereas the presence of ATP-Mg^{2+} was an absolute requirement for the inhibition of vesicle uptake of [^{3}H]-pTA (Lentzen & Philippu, 1977).

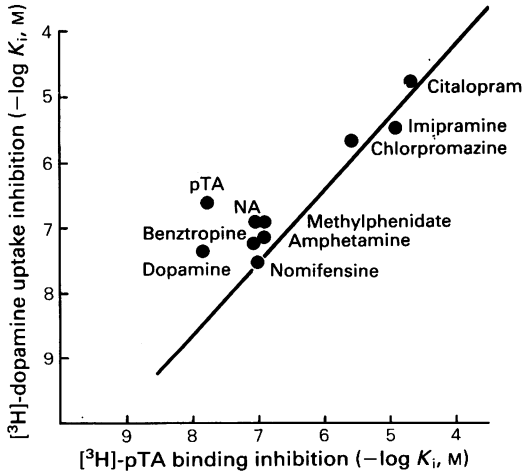

Figure 5 Correlation (r = 0.96, $P < 0.001$) between the affinities of compounds to inhibit [^{3}H]-*para*-tyramine ([^{3}H]-pTA) binding to rat striatal membranes and [^{3}H]-dopamine uptake into rat striatal synaptosomes. The K_i values for inhibition of [^{3}H]-pTA binding were taken from Table 3, and those for inhibition of [^{3}H]-dopamine uptake from Javitch *et al.* (1983) and Richelson & Pfenning (1984). NA = (−)-noradrenaline.

Nevertheless, the present results do not indicate the existence of a central tyramine receptor, instead they suggest that [^{3}H]-pTA associates with a dopamine receptor. There are several results which support this hypothesis: first of all, the equipotency of dopamine, pTA and mTA at displacing [^{3}H]-pTA binding (Table 3). Secondly, unilateral lesion of the nigro-striatal pathway severely decreased the number of striatal [^{3}H]-pTA binding sites (Table 4) and the density of striatal [^{3}H]-dopamine (Leff & Creese, 1983), or [^{3}H]-N, n-propylnorapomorphine (Hall *et al.*, 1983) binding to D_3-type dopamine receptors (Seeman, 1982). Finally, pTA binding sites were highly concentrated in typically dopaminergic brain regions (Table 2). In fact, pharmacological characterization indicated apparent similarities between pTA and D_3 (now D_1^{high}) (Seeman, 1982; Seeman *et al.*, 1985) – binding sites, inasmuch as pTA sites were well recognized by the D_3-agonist dopamine (K_i approx. 10 nM), but only poorly, or not, identified (K_i values in the micromolar range) by several dopamine antagonists known to label D_1- and D_2-receptors (Table 3). Furthermore, both [^{3}H]-pTA-, and [^{3}H]-dopamine-labelled D_3-sites were presynaptically located, as indicated by the loss in binding following denervation of dopamine terminals. However, even though dopamine potently (IC_{50} = 18 nM) displaced specifically bound [^{3}H]-pTA, the binding of [^{3}H]-dopamine to calf (Burt *et al.*, 1976), and rat (Heikkila *et al.*, 1983) striatal membranes was poorly (IC_{50} approx. 10 μM) displaced by pTA. Furthermore, the dopamine agonists apomorphine and pergolide had low affinity (IC_{50} 3–9 μM) for [^{3}H]-pTA sites (Table 3).

All these discrepancies seem to imply that pTA does not interact with a classic dopamine receptor, but rather it labels a site functionally related to the transport of dopamine. Strong support for this hypothesis comes from evidence that the potencies of

various drugs in competing for [^{3}H]-pTA binding to rat striatal membranes correlated closely ($r = 0.96$, $P < 0.001$) with their potencies at inhibiting [^{3}H]-dopamine uptake into striatal synaptosomes (Figure 5). Furthermore, the binding process of [^{3}H]-pTA was Na^+- and temperature-dependent, and sensitive to transport inhibitors such as ouabain and dinitrophenol (Table 1). Finally, unilateral lesion of the nigro-striatal pathway, a procedure that in this laboratory (Raiteri *et al.*, 1985) and elsewhere (Janowsky *et al.*, 1985) has been proved to decrease by 90% the uptake of [^{3}H]-dopamine by rat striatal synaptosomes, reduced by approx. 80% the number of striatal [^{3}H]-pTA binding sites (Table 4).

Depletion of dopamine stores by acute administration of reserpine markedly decreased the number of striatal [^{3}H]-pTA binding sites (Table 5), thus suggesting that the loss of binding following the nigro-striatal lesion (Table 4) was due to the associated depletion of dopamine stores, as well as degeneration of nerve endings. Reserpine *in vitro* also potently inhibited the binding of [^{3}H]-pTA (Table 3). Since reserpine is a putative marker for the dopamine transporter in synaptic vesicles (Near & Mahler, 1983), and the transport of dopamine to any residual intact synaptosomes was highly unlikely due to the disrupting procedure used to prepare brain membranes, a vesicular location of [^{3}H]-pTA binding sites can be hypothesized. More difficult is to evaluate precisely whether pTA interacts with the dopamine recognition site of the vesicular uptake mechanism, or other allosterically linked sites. The guanyl nucleotides GTP, GDP and GMP inhibited the binding of [^{3}H]-pTA (Table 1), thus reflecting nucleotide-provoked inhibition of [^{3}H]-dopamine, [^{3}H]-apomorphine and [^{3}H]-ADTN binding to striatal membranes (Creese *et al.*, 1979; Nomura *et al.*, 1982), possibly as a result of non-competitive inhibition of the binding process (Bacopoulos, 1984).

Failure of ATP to affect the binding of [^{3}H]-pTA (Table 1), in contrast to its reported ability, *in vitro*, to stimulate energy-dependent accumulation of catecholamines and pTA into synaptic vesicles (Toll *et al.*, 1977; Lentzen & Philippu, 1977; Near & Mahler, 1983), suggests that [^{3}H]-pTA was associated, at least partially, at a site located on the outer surface of the vesicle, perhaps the dopamine carrier, itself. Furthermore, reserpine markedly inhibited the binding of [^{3}H]-pTA in the absence of ATP and/or Mg^{2+} ions, whereas the presence of ATP-Mg^{2+} was required in order to inhibit the vesicle uptake of pTA (Lentzen & Philippu, 1977).

This apparent independence of [^{3}H]-pTA binding from energy-linked events contrasted with the additional finding that ouabain, a $Na^+/K^+/Mg^{2+}$-ATPase inhibitor (Bogdanski *et al.*, 1968), and dinitrophenol, an uncoupler of oxidative phosphorylation, decreased the number of [^{3}H]-pTA binding sites (Table 1). Creation of a proton gradient across membranes was also required for binding of [^{3}H]-pTA.

Energy-dependent binding might represent pTA carried through the membrane of intact vesicles: this possibility was supported by the finding that preincubation of striatal suspensions with saponin, which increases the permeability of the vesicle membrane and releases accumulated transmitters (Near & Mahler, 1983), almost abolished the binding of [^{3}H]-pTA (Table 1).

The existence of two components in the reserpine-induced inhibition of the binding process of [^{3}H]-pTA might reflect energy-dependent and energy-independent binding of pTA (Figure 4).

In conclusion, the present results show that in rat striatal membranes, pTA labels a presynaptic site most probably related to the transport of dopamine in synaptic vesicles.

The striking similarities existing between the binding processes of [^{3}H]-pTA and [^{3}H]-dopamine, and the uptake of [^{3}H]-dopamine, support the stimulating hypothesis of LaBella (1985), i.e. that neurotransmitter uptake sites may comprise subtypes of recognition (receptor) sites, and that uptake *per se* represents the composite internalization of the mediator and its corresponding recognition sites. However, the suggestion that pTA displays direct receptor-stimulating activity through specific tyramine receptors of a specific *p*-tyraminergic neuronal system (Stoof *et al.*, 1976; Petrali, 1977) was not corroborated by our results.

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