Regulation of the synthesis and metabolism of striatal dopamine after disruption of nerve conduction in the medial forebrain bundle

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1 After physical (knife-cut) or chemically-mediated (tetrodotoxin 300 nM, 1.5 μ l; 1.0 μ l min⁻¹) interruption of nerve conduction in the nigrostriatal tract, there was a marked increase in the synthesis and metabolism of dopamine in the isolated dopaminergic nerve terminals of the striatum. The effect peaked at 4 h post-transection, at which time 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were increased by 300% and 700% respectively (DOPAC: 27 ± 13 vs 80 ± 17 nmol g⁻¹; HVA: 6.66 ± 3.57 vs 54 ± 18 nmol g⁻¹). The increases in dopamine content and metabolism are secondary to an increase in the rate of synthesis on the lesioned side, versus the intact, control side.

2 In both experimental situations, haloperidol (1.0 mg kg⁻¹, i.p.) retained its known ability to induce a significant increase in DOPAC and HVA in the striatum, despite the interruption of nerve conduction in the nigrostriatal tract.

3 Six days after cutting the left nigrostriatal tract, dopamine in the left striatum was reduced to <5% of the control value, and DOPAC and HVA were not detectable. In the denervated, left striatum, the synthesis of dopamine (from injected L-DOPA), and its metabolism to DOPAC and HVA, occurred to the same degree as in the intact right side. In these DOPA-treated rats, haloperidol (1.0 mg kg⁻¹, i.p.) caused a further increase in DOPAC and HVA in the intact striatum, but not in the denervated striatum.

4 Under non-stressful conditions, using a combination of anaesthetic treatments, electrical stimulation (400 μ A, 0.4 ms, 15 Hz, 15 min) of the nigrostriatal tract did not increase DOPAC or HVA in the striatum on the stimulated side.

5 It is concluded (a) that there is a significant presynaptic, and/or local circuit mechanism capable of activating the synthesis and metabolism of dopamine in the isolated, striatal, dopaminergic nerve terminals. Furthermore, haloperidol can act directly on the striatal, dopaminergic nerve terminal, to cause an increase in the synthesis and metabolism of striatal dopamine. (b) After degeneration of the striatal dopaminergic nerves, the denervated striatum retains the ability to synthesize (from L-DOPA) and metabolize dopamine, to the same degree as the intact, innervated, contralateral striatum. (c) When stress is minimized, and release of dopamine is induced by electrical stimulation of the medial forebrain bundle, the catabolism of dopamine (to DOPAC and HVA) during the release-uptake cycle may not be a significant factor under physiological conditions. (d) When dopamine synthesis is increased in the striatum, the normal blood concentration of tyrosine is adequate to sustain the increased synthesis, and precursor availability is not a limiting factor. (e) These results suggest that some of the basic concepts about the neurochemical/neurophysiological regulation of monoaminergic neurones may require further re-evaluation.

Introduction

The concepts that now guide our thinking about the regulation of transmitter synthesis and metabolism were formulated during the past 25 years (Costa & Meek, 1974). For chemical transmission to be a viable means of information transfer in the central nervous system (CNS), mechanisms should exist to prevent transmitter depletion, especially during periods of increased transmitter demand, e.g., during episodes of increased neuronal firing. Two general schemes have been proposed for monoaminergic neurones. The first is reuptake (Iversen, 1971; 1973). According to this view, most of the released neurotransmitter is resequenced into the monoaminergic nerve terminal by an active uptake process. Based on studies of noradrenergic, sympathetic nerve terminals in the heart, Iversen (1971) estimated that the reuptake mechanism is capable of clearing the synaptic cleft of the released noradrenaline within milliseconds. This finding does not of itself prove that resequenced dopamine is conserved, since it has been suggested that dopamine may be significantly catabolized by monoamine oxidase (MAO) following reuptake

(Westernik & Kikkert, 1986). The question of whether, in the CNS, the resequenced dopamine re-enters the calcium-dependent pool of releasable transmitter and is re-released, is not fully resolved. The second scheme is related to activation of the synthetic enzymes (particularly tyrosine hydroxylase) during increased nerve firing. It has been reported that short-term (seconds to minutes) activation results in a decrease in the K_m of tyrosine hydroxylase for both the precursor (tyrosine) and the pterin cofactor (Murrin *et al.*, 1976). Longer-term transmitter release induced by stress, results appropriately in V_{max} changes. These latter results have been obtained with the adrenal medulla. The published data indicate that gene activation and the associated transcription and translation mechanisms are involved, and occur prior to the increase in V_{max} (Chuang & Costa, 1974; Guidotti & Costa, 1977; Guidotti *et al.*, 1978; Kumakura *et al.*, 1979). However, a stress-induced increase in the synthesis and metabolism of DA has not so far been equivocally demonstrated in the striatum.

Transmitter levels tend not to change significantly under physiological conditions, even during periods of increased release. Therefore, implicit in the idea of increased synthesis, is the notion of increased transmitter catabolism associated with increased release. This phenomenon has been demonstrated under several experimental conditions. Indeed, so generally

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accepted is the association of increased cell firing, increased release and increased metabolism, that increased metabolism is often used as an index of increased firing and increased release (Commissiong, 1985a). Another corollary of the association of increased firing with increased synthesis is the problem of availability of precursor required for an increase in synthesis. The results obtained suggest that when striatal dopamine synthesis is increased, the normal blood concentration of tyrosine is adequate to accommodate the increased synthesis. Raising blood concentration of tyrosine, by injecting tyrosine, does not cause a further increase in synthesis.

Lastly, there is the question of the exact mechanisms by which firing of the nigrostriatal dopaminergic cells is regulated. One of the proposed mechanisms is blockade of the postsynaptic dopamine receptor, usually induced experimentally by neuroleptic drugs. One of the accepted, current hypotheses is that blockade of the postsynaptic dopamine receptor induces an increase in dopaminergic cell firing by a negative feedback mechanism. However, the exact mechanisms of the negative feedback regulatory processes have not been determined (Kehr *et al.*, 1972; Costa & Meek, 1974; Garcia-Munoz *et al.*, 1977; Rosaria & Gale, 1984). Within the present context, it has been suggested that neuroleptic-induced stimulation of the synthesis and metabolism of striatal dopamine is also partly mediated by blockade of the presynaptic D_2 autoreceptors, activation of which normally inhibits synthesis.

In recent years, some authors have suggested that our understanding of the control processes in monoaminergic neurones has been overstated (Commissiong, 1985a; Kuhn *et al.*, 1986; Westerink & Kikkert, 1986). The present experiments were done to reassess (a) the role of local and/or presynaptic mechanisms, (b) increased neuronal activity and precursor availability in the control of the synthesis and metabolism of dopamine in the striatum. The results obtained suggest that under some conditions, released dopamine may be highly conserved, and that *de novo* synthesis and metabolism may be minimal.

Methods

The experiments were done on female Sprague Dawley rats, 250 g. The left nigrostriatal tract was manipulated in three main ways.

(1) By physical knife-cut

A 1.5 mm wide knife, prepared from a single-edge razor blade was mounted in the electrode holder, angled at 70° , of a rat stereotaxic device (SM-15, Narishige Scientific Instrument Company, Tokyo, Japan). The site of insertion was to the left, just lateral (0.5 mm) and caudal to the bregma. The total width of the cut was about 4.5 mm, and it extended to the base of the brain. The hippocampus was avoided by the use of the above coordinates. The procedure resulted in the almost complete severing of the nigrostriatal tract, and a corresponding reduction ($>95\%$) of dopamine in the striatum after 6 days, (Figure 3). The two main metabolites of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were reduced to undetectable levels.

(2) Microinfusion of tetrodotoxin

The tip of a 10 μ l Hamilton syringe was modified to a rounded, tapering, edge. The stereotaxic coordinates used were: AP 2.80 mm; H: 3.0 mm and L: 1.4 mm, and 1.5 μ l of a 3.0×10^{-7} M solution of tetrodotoxin (TTX) (Narahashi *et al.*, 1967) was slowly infused ($1.0 \mu\text{l min}^{-1}$) into the left medial forebrain bundle (MFB). This dose of TTX caused a 50% reduction in the evoked potential measured in the MFB at 4 mm rostral to the infusion site of TTX, for the 4 h duration of the experiment. TTX (1.5 μ l, infused at $1.0 \mu\text{l min}^{-1}$) at a concentration of 1.0×10^{-6} M was needed to attenuate the evoked potential in the MFB completely, but caused no

further, significant increase in the metabolism of dopamine. The MFB was stimulated at 1.2 mm caudal to the infusion site of TTX. The tip of the needle was left in place for a further minute after the infusion, and then was slowly withdrawn incrementally. A test injection of 1.5 μ l of a 0.5% Brilliant Blue dye, followed by a transcardiac perfusion, and freezing and cutting of the brain at 48 μ m thick sections, indicated that the tip of the syringe could be reproducibly placed in the MFB.

(3) Electrical stimulation

A concentric bipolar stimulating electrode (SNEX-100, Korf Instrument Company) was used. The stereotaxic coordinates were: AP: 2.80 mm; H: 3.00 mm; L: 1.4 mm. A grass 48 stimulator was connected to a Grass constant current unit (CCU). The electrode was connected to the output of the CCU which delivered constant current biphasic pulses of 400 μ A, and 0.4 ms duration. This arrangement prevented variation in stimulation intensity during the experiment due to variation in the resistance of the tip of the stimulating electrode. Stimulation was at 15 Hz, for 15 min.

Anaesthetics

Brietal (50 mg kg $^{-1}$, i.p.), Urethane (1.5 g kg $^{-1}$, i.p.), chloral hydrate (400 mg kg $^{-1}$, i.p.) and halothane (2.5%) were used in different experiments. In the results illustrated in Table 6, the animals were anaesthetized initially with halothane, and then injected with Brietal, Urethane or chloral hydrate. In this experiment, designed to test the contribution of stress on increased dopamine metabolism in the striatum, each animal was isolated individually in a 1 cubic foot rat box for 15 min, and anaesthesia was induced by a brief exposure to halothane (2.5%). The animals were then quickly removed from the rat box and injected with Brietal or Urethane or chloral hydrate. The animals recovered from halothane very rapidly (within 1 min). Therefore during the electrical stimulation of the MFB to evoke the release of dopamine, anaesthesia was maintained by Brietal or Urethane or chloral hydrate, but never by halothane. In the companion experiment (see Table 7), the animals were injected with Brietal, without prior isolation or without being initially anaesthetized with halothane.

Neurochemical analysis

A Finnigan 4000 gas chromatographic-mass spectrometric system was used. For the analysis of dopamine, DOPAC and HVA the tissues were homogenized in a 100 μ M ascorbic acid – 0.1 N HCl solution at a 1:10 w/v ratio, with a Kontes micro ultrasonic cell disrupter (Cole-Parmer, Chicago, ILL.). The homogenate was centrifuged at 35,000 g at 0°C , for 25 min. A more complete precipitation of protein was obtained by adding 6.0 N HCl (1/10 of the homogenizing volume) to the supernatant, allowing the tubes to stand on ice for 15 min, and then centrifuging again at 35,000 g at 0°C , for 30 min. For the analysis of dopamine, 50 μ l of the supernatant was transferred to a 1.0 ml microflex vial, and 257 pmol [$^2\text{H}_4$]-dopamine in 10 μ l of 1.0 N HCl was added as the internal standard. For the analysis of DOPAC and HVA, 100 μ l of the supernatant was transferred to a 1.0 ml microflex vial, and 290 pmol [$^2\text{H}_5$]-DOPAC and 214 pmol [$^2\text{H}_5$]-HVA each in 10 μ l 1.0 N HCl were added. The samples were dried in a speed-vac concentrator (Savant instruments Inc., Farmingdale, N.Y.). Dopamine was acylated with pentafluoropropionic anhydride (PFPA), 100 μ l, plus 20 μ l of ethyl acetate at 60°C for 30 min. DOPAC and HVA were esterified with hexafluoroisopropanol (HFIP), 20 μ l, and acylated with PFPA, 100 μ l, at 70°C for 90 min. The derivatized samples were dried with ultra pure N_2 , reconstituted in 10 μ l of dehydrated ethyl acetate prior to being injected into the GC-MS system (Commissiong *et al.*, 1978).

For the analysis of γ -aminobutyric acid (GABA), 2.6 nmol [$^2\text{H}_6$]-GABA in 10 μ l of 1.0 N HCl was added to 100 μ l of the supernatant, and the mixture applied to a 1.0 cm high Dowex

Table 1 Identification of biological dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) from ion ratio and GC retention time data

Criteria	Dopamine	DOPAC	HVA
<i>m/z</i> :	428/387	415/387	478/331
Authentic	22.4 ± 1.94(4)	2.24 ± 0.12(5)	2.19 ± 0.18(5)
Biological	22.0 ± 2.58(29)	2.32 ± 0.15(29)	2.15 ± 0.17(29)
Retention time (min):			
Authentic	1.37 ± 0.02(4)	1.58 ± 0.02(4)	3.14 ± 0.02(4)
Biological	1.37 ± 0.03(4)	1.58 ± 0.03(4)	3.13 ± 0.02(4)

Dopamine: O-17 3%; 165°C

DOPAC and HVA: OV-17 3%; 120°C

Dopamine, DOPAC and HVA were identified from the statistical identity of the ratio of two ions from each compound, and from their GC retention times. In each case there was no statistical difference in the values obtained for the authentic compounds vs the putative biological compounds from the striatum. All analyses were done isothermally (dopamine: 165°C, DOPAC and HVA: 120°C) using OV-17 3% (dopamine, DOPAC, HVA) as the stationary phase.

cation exchange resin chromatographic column (Pasteur pipette). GABA was subsequently eluted with 800 µl 3.0N ammonia solution. The eluate was collected in 1.0 ml microflex vials, dried in a speed-vac concentrator and derivatized with 25 µl HFIP and 100 µl PFFA at 60°C for 60 min. The derivatized samples were dried with N₂ and reconstituted in 10 µl of ethylacetate prior to being injected into the GC-MS system.

The gas chromatographic (GC) analysis of dopamine was done on a 6 ft glass column, packed with OV-17 3% coated on Gas Chrom Q-HP, 80-100 mesh (chromatographic Specialties, Ontario, Canada). The analysis of dopamine was done isothermally at 165°C. DOPAC and HVA were analyzed on the same GC column used for dopamine, but at 120°C. GABA was analyzed on a 6 ft long glass column, packed with OV-210 3%, at 120°C.

The ions (*m/z*) monitored were: dopamine: 387, 428, [²H₄]-dopamine: 431; DOPAC: 387, 415; [²H₅]-DOPAC: 420; HVA: 331, 478; [²H₅]-HVA: 483; GABA: 190, 204, 232, [²H₅]-GABA: 238. The identities of the putative biological compounds analyzed were based on (1) ion ratios, (2) GC retention times comparisons, see Table 1.

All deuterated compounds were obtained from Merck Frost, Pointe Claire, Quebec, Canada.

Results

Synthesis in the isolated dopaminergic terminals

The results illustrated in Figure 1 show that after the dopaminergic axons are cut, the isolated dopaminergic nerve terminals can still synthesize and metabolize dopamine for 24 h afterwards. The effect peaked at 4 h, and returned to near control values by 6 h after the lesion. The concentrations of DOPAC and HVA were increased by factors of >3 and >7 respectively at 4 h, in comparison with their control values at 0 h. Blockade of nerve conduction in the nigrostriatal fibres, by the microinfusion of TTX into the MFB, caused a qualitatively similar effect as that obtained with the physical knife-cut procedure (Figure 2). The observed increase in dopamine, DOPAC and HVA after the interruption of nerve conduction by either technique is due to a real increase in the rate of synthesis on the lesioned side, versus the intact, control side, and not merely to an accumulation of dopamine, DOPAC and HVA in the nerve terminal, secondary to a cessation of release, induced by the experimental blockade of neurotransmission. In experiments in which the decarboxylase inhibitor 3-hydroxybenzylhydrazine hydrochloride (NSD-1015, 200 mg kg⁻¹ i.p.; 100 mg kg⁻¹ given just prior to either the lesion or the infusion of TTX, and a further 100 mg kg⁻¹ 2 h later) was used and the striata analyzed at 4 h after the

Figure 1 Changes in dopamine (a), 3,4-dihydroxyphenylacetic acid (DOPAC) (b) and homovanillic acid (HVA) (c) at 0-24 h after cutting the left nigrostriatal tract. Rats were anaesthetized with Brietal (50 mg kg⁻¹ i.p.), and the left nigrostriatal tract completely cut under stereotaxic guidance. At 0, 1, 2, 4, 6, 12 and 24 h later, the animals were killed by decapitation, the striata quickly removed and frozen, and subsequently analyzed for dopamine, DOPAC and HVA. At 4 h post-lesion, there was a significant increase in DOPAC and HVA ($\times 4$ and $\times 7$ times respectively). At 12 h post-lesion there was a significant increase in dopamine but DOPAC and HVA had returned to control values. Values are mean with s.d. shown by vertical lines ($n = 5$). Open symbols, left side; closed symbols, right side. (a) Solid asterisk; $P < 0.05$ vs dopamine concentration at 12 h on the right side (b and c) open asterisk; $P < 0.001$ vs DOPAC and HVA concentrations respectively at 4 h on the right side.

lesion, the concentration of DOPA was 3.5 times higher on the left, lesioned side, compared with the intact, control side (Commissong & Slimovitch, 1987). This activation of tyrosine hydroxylase in the striatum following blockade of nerve conduction in the nigrostriatal fibres has been demonstrated previously by other authors (Carlsson *et al.*, 1972; Morgenroth *et al.*, 1976; Nowycky & Roth, 1978). It is likely that the increase in synthesis is underestimated, since transport of DOPAC and HVA from the striatum is not taken into account.

The results shown in Table 2, demonstrate that haloperidol retains its known ability to increase the concentration of DOPAC and HVA significantly in the striatal dopaminergic nerve terminals, after they were isolated from the cell bodies.

Figure 2 The effect tetrodotoxin (TTX) on the synthesis and metabolism of dopamine in the striatum of the rat. Rats were anaesthetized with Brietal (50 mg kg⁻¹ i.p.) and mounted in the stereotaxic frame. TTX (300 nM; 1.5 µl; 1.0 µl min⁻¹) was infused into the left MFB; 4 h later the animals were decapitated and the striata removed and analyzed for dopamine (a), 3,4-dihydroxyphenylacetic acid (b) and homovanillic acid (c). The results obtained are qualitatively similar to those obtained after a physical interruption of the nigrostriatal axons by a knife-cut technique (see Figure 1). Values are mean with s.d. shown by vertical lines ($n = 5$). Open columns, unoperated controls; lined columns, saline-treated; stippled columns, tetrodotoxin treated. L: left side; R: right side. * $P < 0.01$ vs any of the other five measures in the group.

Table 2 The effect of haloperidol on striatal dopamine metabolism after complete transection of the left nigrostriatal tract

	Unoperated vehicle		Unoperated haloperidol		Lesioned vehicle		Lesioned haloperidol	
	Left	Right	Left	Right	Left	Right	Left	Right
Dopamine	56 ± 7	65 ± 9	55 ± 6	51 ± 9	110 ± 9 ^a	61 ± 8	98 ± 17	54 ± 5
DOPAC	18 ± 3	19 ± 2	50 ± 6	59 ± 14	20 ± 3	20 ± 3	29 ± 4 ^b	70 ± 13 ^d
HVA	6.3 ± 1.1	8.0 ± 1.2	19 ± 3	21 ± 4	4.4 ± 1.5 ^a	6.8 ± 1.6	10.2 ± 4.8 ^c	20 ± 2

The effect of haloperidol on the synthesis and metabolism of dopamine in the striatum of rats at 10 h after transection of the left nigrostriatal tract. Rats were anaesthetized with Brietal (50 mg kg⁻¹, i.p.) and the left nigrostriatal tract was lesioned under stereotaxic guidance; 10 h later, the animals were treated with haloperidol (1.0 mg kg⁻¹ i.p., 1 h), and then killed by decapitation. The striata were quickly removed, frozen, and subsequently analyzed for dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). The statistically significant reduction of HVA on the left, lesioned side in the lesioned/vehicle group was unexpected and difficult to explain.

Values are nmol g⁻¹ ± s.d. (n = 5)

^a: P < 0.01 vs L: Unoperated/Vehicle

^b: P < 0.01 vs L: Lesioned/Vehicle or L: Unoperated/Vehicle

^c: P < 0.01 vs L: Lesioned/Vehicle or L: Unoperated/Vehicle

^d: P < 0.02 vs R: Unoperated/Haloperidol

For the analysis of variance for the L side:

Dopamine: F(7, 32) = 35.6; P < 0.01

DOPAC: F(7, 28) = 32.3; P < 0.01

HVA: F(7, 30) = 27.4; P < 0.01

This effect of haloperidol is, however, attenuated, versus its normal effect on the intact striatum. It should be noted, that at 10 h after lesioning the nigrostriatal tract, the nerve terminal had previously (0–6 h, peaking at 4 h) been through a

Figure 3 The depletion of dopamine (a), 3,4-dihydroxyphenylacetic acid (DOPAC) (b) and homovanillic acid (HVA) (c) in the chronically-denervated left striatum of the rat. Rats were anaesthetized with Brietal (50 mg kg⁻¹, i.p.) and the left nigrostriatal tract was cut under stereotaxic guidance. Six days later the animals were killed by decapitation, the left and right striatum were quickly removed and frozen, and subsequently analyzed for dopamine, DOPAC and HVA. Dopamine was just detectable on the lesioned (L) side, and DOPAC and HVA (a, b and c respectively) were below the limits of detection. Values are mean with s.d. shown by vertical lines (n = 5). L: left striatum; R: right striatum; LES: lesioned; UNLES: unlesioned.

cycle of markedly enhanced synthesis and metabolism of dopamine. Whether this is responsible for the observed, attenuated effect of haloperidol is not certain. The experiment was designed in this way, so that the analyses could be done when the concentrations of DOPAC and HVA were in a steady state, or changing at a very low rate. When nerve conduction in the nigrostriatal pathway was blocked by the microinjection of TTX into the MFB, haloperidol still caused a statistically significant increase in both DOPAC and HVA in the striatum (Table 3). In this case haloperidol was injected at 20 min after the infusion of TTX, when the animals had recovered from the anaesthetic. This allowed enough time for the diffusion of TTX and blockade of the voltage-dependent sodium channels (Koizumi *et al.*, 1967). The rats were killed 1 h later, by which time the TTX-induced increase in dopamine, DOPAC and HVA was negligible (see Figure 1). Therefore, the results for the left striatum shown in Table 3 are due to the action of haloperidol on striatal dopaminergic terminals, with nerve conduction blocked in the left MFB. The results show that the effect of haloperidol in these recently-anaesthetized animals is attenuated, versus the effect normally obtained in unoperated, control animals. More importantly, in this case, unlike the knife-cut experiments (see Table 2), the effects on the left and right sides are identical, suggesting that the effect of haloperidol on the synthesis and metabolism of dopamine in the striatum, may be entirely presynaptic.

Chronic denervation

The effect of chronic denervation (cutting the nigrostriatal dopaminergic fibres six days previously) on dopamine,

Table 3 The effect of haloperidol on striatal dopamine metabolism after the microinfusion of tetrodotoxin (TTX) into the left nigrostriatal tract

	Unoperated vehicle		Unoperated haloperidol		TTX vehicle		TTX haloperidol	
	Left	Right	Left	Right	Left	Right	Left	Right
Dopamine	63 ± 10	66 ± 6	48 ± 6	46 ± 6	75 ± 20	75 ± 9	61 ± 15	61 ± 8
DOPAC	31 ± 7	28 ± 8	118 ± 49	108 ± 25	29 ± 14	33 ± 7	59 ± 14 ^b	61 ± 13 ^c
HVA	7.0 ± 1.3	6.9 ± 1.2	23 ± 2	22 ± 4	6.2 ± 2.6	6.0 ± 2.0	14 ± 3 ^c	15 ± 1.7 ^f

The effect of TTX and haloperidol on the synthesis and metabolism of dopamine in the striatum of the rat. Rats were anaesthetized with Brietal (50 mg kg⁻¹ i.p.) and TTX (1.5 µl: 300 nM) was microfused (1.0 µl min⁻¹) into the left MFB under stereotaxic guidance. After recovery from the anaesthetic (about 20 min) the animals were injected with haloperidol (1.0 mg kg⁻¹, i.p.; 1 h), and then decapitated. The striata were quickly removed and frozen, and subsequently analyzed for dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). There was a significant (P < 0.05) increase in both DOPAC and HVA on the left (L) side in the TTX/Hal group vs the concentration in the L: TTX/Vehicle group. The dose of TTX used (1.5 µl, 3.0 × 10⁻⁷ M), reduced the evoked potential in the MFB to < 50% within a few minutes of being applied. This degree of suppression of nerve conduction remained effective for up to 4 h.

Values are nmol g⁻¹ ± s.d. (n = 5)

^b: P < 0.01 vs L: TTX/Vehicle or L: Unoperated/Vehicle

^c: P < 0.01 vs L: TTX/Vehicle or L: Unoperated/Vehicle

^d: P < 0.01 vs R: TTX/Vehicle or R: Unoperated/Vehicle

^e: P < 0.01 vs R: TTX/Vehicle or R: Unoperated/Vehicle

For the analysis of variance for the L side:

Dopamine: F(7, 31) = 3.38; P < 0.05

DOPAC: F(7, 29) = 6.89; P < 0.01

HVA: F(7, 32) = 6.19; P < 0.01

Table 4 The effect of haloperidol on the metabolism of dopamine (synthesized from injected L-DOPA) in the chronically-denervated rat striatum

Conditions		Dopamine		DOPAC		HVA	
		Left	Right	Left	Right	Left	Right
Unles	HAL ⁻ DOPA ⁻	35 ± 4	44 ± 12	21 ± 5	23 ± 9	7.7 ± 1.6	7.7 ± 1.4
Les	HAL ⁻ DOPA ⁻	2.5 ± 0.8	51 ± 11	Det	24 ± 5	Det	4.7 ± 1.2
Les	HAL ⁺ DOPA ⁻	1.2 ± 0.1	44 ± 3	3.1 ± 1.3	80 ± 18	Det	22 ± 1
Les	HAL ⁻ DOPA ⁺	10 ± 4	79 ± 9	218 ± 45	176 ± 43	45 ± 14	37 ± 8
Les	HAL ⁺ DOPA ⁺	10 ± 7	49 ± 5*	199 ± 50	350 ± 111*	43 ± 4	50 ± 1*

In lesioned (Les) animals, the left nigrostriatal tract (L-NST) was cut as described in the Methods section. The experiment was done 6 days later, after the striatal dopaminergic nerves had degenerated (Figure 3). Haloperidol (1.0 mg kg⁻¹, i.p.) was injected at 0 min, L-DOPA (100 mg kg⁻¹, i.p.) at 15 min, and the animals killed at 75 min. In the lesioned animals treated with L-DOPA (Les/Hal⁻/DOPA⁺ and Les/Hal⁺/DOPA⁺ groups), the production of DOPAC and HVA in the denervated left striatum was as great as in the intact right striatum. However, haloperidol induced a further increase in DOPAC and HVA in the intact right striatum, but not in the denervated left striatum.

Det: detectable only. Values are nmol g⁻¹ ± s.d. (n = 5).

* P < 0.05 vs the right side of the Les/Hal⁻/DOPA⁺ group

Unles: unlesioned; Les: lesioned.

Hal⁻: not treated with haloperidol; Hal⁺: treated with haloperidol.

DOPA⁻: not treated with DOPA; DOPA⁺: treated with DOPA.

Table 5 The effect of tyrosine on the lesion-induced enhanced synthesis and metabolism of dopamine in the striatum of the rat

Condition		Dopamine	DOPAC	HVA
Unles + Veh	L	60 ± 8	20 ± 7	6.7 ± 1.4
	R	62 ± 3	18 ± 2	6.9 ± 1.0
Unles + Tyr	L	61 ± 4	22 ± 6	6.3 ± 0.4
	R	67 ± 3	23 ± 4	6.6 ± 1.2
Les + Veh	L	117 ± 8	57 ± 12	22 ± 2
	R	68 ± 2	24 ± 7	7.3 ± 1.1
Les + Tyr	L	105 ± 4*	51 ± 6	21 ± 2
	R	69 ± 4	19 ± 3	6.5 ± 0.8
Left side ANOVA	F(3, 16) =	9.64	15.7	23.6
	P	0.01	0.01	0.01

Rats were anaesthetized with Brietal (50 mg kg⁻¹, i.p.) and the left MFB cut under stereotaxic guidance; 2.5 h later, the animals were injected with tyrosine (150 mg kg⁻¹ i.p.), and killed 1.5 h later, i.e. at 4 h after the lesion, when the lesion-induced metabolism of dopamine is at its peak (see Figure 1). The striata were rapidly removed, frozen, and subsequently analyzed for dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). The injected tyrosine caused a significant (P < 0.05) reduction in the lesion-induced synthesis of dopamine, and also caused a small but non-significant reduction in the lesion-induced increase in DOPAC and HVA.

Values are nmol g⁻¹ ± s.d. (n = 5)

* P < 0.05 vs L: Les + Veh group. Student's unpaired t test.

DOPAC and HVA in the striatum is shown in Figure 3. Dopamine was reduced by >95%, and DOPAC and HVA were reduced to nondetectable levels. However, after the injection of L-DOPA (100 mg kg⁻¹ i.p., 1 h), there was a striking increase in the synthesis and metabolism of dopamine in the chronically denervated striatum (Table 4). The concentrations of DOPAC and HVA increased from undetectable levels to over 200 and 45 nmol g⁻¹ respectively. On the innervated right side, the magnitude of the effect was similar to that on the denervated left side (Table 4). Haloperidol caused a further, statistically significant increase in the metabolism of dopamine in the intact right striatum, but not in the denervated, left striatum (Table 4). Therefore, haloperidol does exert a specific effect on the dopaminergic neurone. The experimental design used does not permit a differentiation between a purely presynaptic/local effect, and an effect based on post-synaptic receptor blockade and subsequent activation of the dopaminergic neurone by a negative feedback mechanism.

Precursor considerations

Experiments were done in acutely denervated and in normal rats to test the effect of administering tyrosine (150 mg kg⁻¹ i.p., 1.5 h) on the synthesis and metabolism of dopamine in the striatum. The results are shown in Table 5. Tyrosine had no effect on the synthesis of dopamine in the intact control right striatum but caused a significant (P < 0.05) reduction in the concentration of dopamine in the denervated, left striatum. An

Table 6 The effect of electrical stimulation on the left nigrostriatal tract, under non-stressful conditions on metabolism of dopamine in the striatum

Conditions	Dopamine		DOPAC		HVA	
	Left	Right	Left	Right	Left	Right
Control (B)	90 ± 2	90 ± 3	21 ± 4	22 ± 4	7.2 ± 1.1	8.3 ± 1.6
Sham Stim (B)	87 ± 7	89 ± 4	26 ± 9	23 ± 7	8.0 ± 2.1	7.4 ± 2.0
Stimulated (CH)	88 ± 9	86 ± 24	18 ± 3	18 ± 3	8.8 ± 2.0	8.4 ± 2.4
Stimulated (B)	92 ± 8	86 ± 12	22 ± 2	19 ± 4	7.9 ± 0.7	7.5 ± 1.4
Stimulated (U)	97 ± 12	102 ± 5	27 ± 6	23 ± 4	7.7 ± 1.4	7.4 ± 1.0
F	0.80	0.06	1.99	0.59	0.48	0.33
P	NS	NS	NS	NS	NS	NS

Values are nmol g⁻¹ ± s.d. (n = 5)

After randomization, each animal was isolated in a 1.0 cubic ft. rat box for 15 min, and anaesthetized with halothane. Anaesthesia was then continued with either chloral hydrate (CH, 400 mg kg⁻¹, i.p.); or Brietal (B, 50 mg kg⁻¹, i.p.) or Urethane (U, 1.5 g kg⁻¹, i.p.). The left nigrostriatal tract was stimulated electrically (400 µA; 15 Hz) for 15 min. The animals were decapitated and the left and right striata removed and analyzed for dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Under these experimental conditions, using ANOVA analysis, there was no significant difference among the members of any group. There was also no significant difference between the left vs right sides for any of the three anaesthetics in any of the four conditions.

Table 7 The effect of electrical stimulation of the left nigrostriatal tract on the synthesis and metabolism of dopamine in the striatum

Conditions	Dopamine		DOPAC		HVA	
	Left	Right	Left	Right	Left	Right
Control (B)	64 ± 10	66 ± 8	18 ± 3	22 ± 5	5.9 ± 1.1	6.5 ± 1.3
Sham Stim (B)	69 ± 3	73 ± 10	18 ± 3	21 ± 1	5.6 ± 0.5	6.7 ± 0.8
Stimulated (B)	101 ± 39	77 ± 6	43 ± 3*	26 ± 9	8.5 ± 1.2	8.5 ± 1.2
<i>F</i>	2.27	2.88	6.12	0.71	7.12	4.43
<i>P</i>	NS	NS	0.05	NS	0.01	0.05

**P* < 0.05 vs the stimulated, right side. Values are nmol g⁻¹ ± s.d. (*n* = 5)

The animals were randomized, and then anaesthetized with Brietal. The left nigrostriatal tract was stimulated electrically (400 µA; 15 Hz) for 15 min. The left and right striata were then removed, and analyzed for dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). ANOVA analysis showed that there was a significant difference among the members of the following groups: DOPAC, left side; HVA: left and right sides. However, in the left vs right comparisons, the only significant difference found was for DOPAC.

ANOVA statistical analysis was done initially (*F*(3, 16) = 9.64; (*P* < 0.01)) followed by an unpaired Student's *t* test.

Electical stimulation

Electrical stimulation (of the MFB) experiments were done to test the relationship between release and metabolism. The left nigrostriatal fibres were stimulated in three groups of rats anaesthetized respectively with chloral hydrate (400 mg kg⁻¹, i.p.), Brietal (50 mg kg⁻¹, i.p.) or Urethane (1.5 g kg⁻¹, i.p.). The unstimulated control and sham-stimulated control groups were anaesthetized with Brietal (50 mg kg⁻¹, i.p.). Each animal used in this experiment was isolated in a rat box for 15 min, and anaesthesia was induced with halothane. Subsequently, the animals were injected with the anaesthetic agents described above. Under these relatively non-stressful conditions, neither chloral hydrate nor electrical stimulation caused an increase in DOPAC and/or HVA (Table 6). However, when the experiment was repeated in the traditional manner, without pre-anaesthetizing the animals with halothane, the more usual increases in DOPAC and HVA were observed (Table 7).

Discussion

Two techniques have been used to interrupt nerve conduction in the nigrostriatal, dopaminergic fibres: physical knife cut of the axons, and specific chemical blockade of the voltage-dependent sodium channels with TTX. In both cases there was a marked increase in dopamine, DOPAC and HVA on the experimental, lesioned side, versus the intact, control side (Figures 1 and 2). The increases in the content of dopamine, DOPAC and HVA are due to an increase in the rate of synthesis of dopamine on the experimental side, versus the intact, control, side. In experiments in which the decarboxylase inhibitor NSD-1015 was used, the accumulation of DOPA, measured at 4 h after the lesion, was 3.5 times greater on the lesioned left side than on the control right side. Therefore, tyrosine hydroxylase (TH) was activated in the striatum on the lesioned, left side, as compared with the control, right side, as has been shown previously (Carlsson *et al.*, 1972; Roth *et al.*, 1974; Morgenroth *et al.*, 1976; Walters and Roth, 1976a; Nowicky & Roth, 1978; Kehr *et al.*, 1982; Wood, 1982; Wood & Richard, 1982). The present results reaffirm the interesting paradox that both increased impulse flow and cessation of impulse flow in the nigrostriatal fibres can cause an activation of TH in the striatum, and an associated increased synthesis/metabolism of dopamine (Nowicky & Roth, 1978). It will be argued (see later) however, that increased impulse flow induced by electrical stimulation may not, in fact, always cause an activation of TH, as has been proposed (Murrin *et al.*, 1976). A question of general concern is the neurochemical viability of the striatum during the long duration of some of

these experiments (Table 2). GABA is a relatively labile transmitter with a fast turnover rate, and can be used to give a useful neurochemical/physiological index of the state of a brain region in which it is found. The results in Figure 4 were obtained from an analysis of the same supernatant used to generate the data in Table 2. GABA was unchanged throughout the 10 h duration of the experiment, suggesting that the striatum may not have been compromised in any significant way during the course of the experiment.

In the subsequent experiments, the two methods of increasing striatal dopamine synthesis were used to study some of the factors that are thought to modulate the synthesis of dopamine. In this regard, the mechanism of action of antipsychotic drugs is one of the most interesting problems. Haloperidol caused a classical dose-dependent increase in DOPAC and HVA in the striatum (Figure 5), and a consistently small, usually non-significant fall in dopamine. The results in Table 2 and 3 show that haloperidol was still effective in increasing the concentration of DOPAC and HVA in the striatum after

Figure 4 The effect of acute (10 h) transection of the MFB on the content of γ -aminobutyric acid (GABA) in the striatum of the rat. The supernatant is from the tissue used to obtain the data illustrated in Table 2. The results show that there was no significant change in the concentration of GABA in any of the 8 groups of tissue analyzed. Values are mean with s.d. shown by vertical lines (*n* = 5). (a) unoperated-vehicle; (b) unoperated-haloperidol; (c) lesioned-vehicle; (d) lesioned-haloperidol. L: left, lesioned side; R: right, intact, control side.

Figure 5 A semi-logarithmic plot of the dose-response effect of the action of haloperidol (Hal) on the concentration of (a) dopamine, (b) 3,4-dihydroxyphenylacetic acid (DOPAC) and (c) homovanillic acid (HVA) in the striatum of the rat. Rats were injected with haloperidol at 0, 0.2, 1.0 and 5.0 mg kg⁻¹ i.p. One hour later, the animals were killed by decapitation, the striata quickly removed, frozen, and subsequently analyzed for dopamine, DOPAC and HVA. Haloperidol consistently caused a reduction (usually non-significant) in the concentration of dopamine and a significant increase in DOPAC and HVA. Values are mean with s.d. shown by vertical lines ($n = 5$). In (a) $F = 2.17$, NS; (b) $F = 29.5$, $P < 0.001$; (c) $F = 52.1$, $P < 0.001$.

blockade of nerve conduction in the MFB. Taken together, these results suggest that haloperidol can act directly on the dopaminergic nerve terminal. Therefore, the results may be unrelated to a striatonigral, long-loop, negative feedback activation of the dopaminergic neurone, as is usually suggested (Carlsson *et al.*, 1972; Iwatsubo & Clouet, 1977; Kilpatrick *et al.*, 1985; Johnson *et al.*, 1986). An alternative explanation is that haloperidol may increase striatal DOPAC and HVA by blocking their transport from the striatum (Westerink & Kikkert, 1986). However, this explanation is unlikely, in view of the results illustrated in Table 4, in which haloperidol increased DOPAC and HVA in the striatum only on the innervated side.

In Table 4, it is also shown that in the denervated striatum (see Figure 3) the synthesis and metabolism of dopamine from injected DOPA were the same as in the innervated control striatum. Therefore, provided the precursor (DOPA) is presented beyond the TH regulatory point, the striatum, minus its dopaminergic nerves, can still synthesize and metabolize dopamine as efficiently as an intact striatum. The decarboxylase enzyme, however, is known to be present in both non-neural (glia, endothelium) and other monoaminergic neurones (Langelier *et al.*, 1972; Jaeger *et al.*, 1973; Hefti *et al.*, 1981; Commissiong, 1985b; El Gemayel *et al.*, 1986). Thus considerable caution should be exercised in the interpretation of dopamine metabolism data, since it is likely that a large fraction of DOPAC and HVA may be non-neural in origin, and therefore

may not be indicative of events in the dopaminergic neurone. Moreover, despite the magnitude of the synthesis/metabolism that ensued after DOPA injection, haloperidol still enhanced the effect, but only in the innervated striatum. One plausible explanation of this effect is a direct action of haloperidol on the synthesis-inhibiting D₂ autoreceptors on the dopaminergic nerve terminal, since haloperidol has been shown to increase tyrosine hydroxylase activity directly, *in vitro*, in a synaptosomal preparation (Christiansen & Squires, 1974). It is surprising that this regulatory mechanism remains active even under conditions of an ongoing excessive synthesis and metabolism of DA (Table 4). This result suggests that there may be stringent, inhibitory mechanisms in the dopaminergic nerve terminal which normally limit the synthesis and metabolisms of DA to a small fraction of the maximum capacity. These results are also of general significance with regard to the therapeutic use of L-DOPA to treat Parkinson's disease. Evidently, the entire CNS must be flooded with high concentrations of dopamine in the hours immediately after ingestion of L-DOPA (see Commissiong, 1985b). Looked at from this perspective, it is surprising that L-DOPA has proven to be a beneficial therapeutic agent and not surprising that it ceases to be effective in a significant fraction of cases after a long period of use.

The increased post-lesion synthesis of dopamine afforded a good experimental test of the idea that when dopamine synthesis is activated, precursor (blood tyrosine concentration) may become a limiting factor. This hypothesis was tested directly (Table 5). Other authors have addressed this question previously (Murrin *et al.*, 1976; Milner & Wurtman, 1986). The results obtained (Table 5), suggests that when striatal dopamine synthesis is increased, blood tyrosine concentration is not a limiting factor. Indeed, the data suggest that *in vivo*, increasing tyrosine concentration may actually inhibit the synthesis (though curiously not the metabolism) of dopamine.

The last and most important aspect of the results described in this paper relate to the fate of released dopamine. The results shown in Table 6 indicate that when stress is minimized, transmitter catabolism (increased DOPAC and HVA) may not be a necessary accompaniment of increased dopamine release following electrical stimulation. These data were obtained from rats that were subjected initially to an additional anaesthetic (halothane) exposure, but the halothane was used only to induce anaesthesia, and the animals recovered rapidly (usually within a minute), if anaesthesia was not maintained by Brietal or Urethane or chloral hydrate. This experimental design was based on the previous evidence (Buhler *et al.*, 1978) that plasma catecholamines increase rapidly in response to the stress of gentle handling. It has been demonstrated that halothane does not interfere with the evoked release of dopamine (Besson *et al.*, 1971; Nicoullon *et al.*, 1977; Zetterstrom *et al.*, 1983). Nevertheless, general anaesthetics have been shown to suppress and enhance synaptic transmission, presumably by an effect on transmitter release (Richards, 1974). On balance, it is unlikely that the present results can be accounted for solely by a halothane-mediated suppression of dopamine release, since at the time the MFB was stimulated electrically, anaesthesia was being maintained by Brietal or Urethane or chloral hydrate. The results also suggest that reuptake is a robust mechanism, and may be adequate to replenish transmitter stores even in cases of greatly enhance release. This conclusion is supported by the observation that when the experiment was done without preventing the stress caused by handling, by the initial use of halothane, some evidence for catabolism was obtained (Table 7).

The general thrust of this paper has been to show that some of the basic ideas that guide our thinking in the interpretation of monoaminergic neurochemical/neurophysiological data may be equivocal. In particular, the use of metabolite measurements as an index of release and by implication, as an index of nerve activity is in all probability an unsound procedure. Recent evidence, based on *in vivo* release data, using the intracerebral dialysis technique, suggests that dopamine

release and metabolism (indicated by DOPAC and HVA) can be varied independently under some experimental conditions (Imperato & Dichiaro, 1985). It has been shown that different mechanisms underlie the effects of neuroleptics on dopamine release and metabolism. Intraneuronal synthesis and metabolism, unrelated to release, could be another important, confounding factor in the interpretation of data. Presynaptic and heterosynaptic mechanisms appear to play significant roles in the regulation of the synthesis/metabolism of dopamine in the striatum (Walters & Roth, 1976b; Leviel *et al.*, 1979; Ganon & Buda, 1985; Kalser, 1985; Laduron, 1985; Nissbrandt *et al.*, 1985). Several reports have shown that the mesotelencephalic dopaminergic neurones constitute a variety of heterogeneous subgroups, and that they are regulated differently (Demarest & Moore, 1979; Bannon *et al.*, 1982; Bannon & Roth, 1983; Roth, 1984; DiChiara & Imperato, 1985). One significant

recent advance is the ability to monitor dopaminergic neuronal activity in transplants with apparently no afferent connections, and therefore to test the effects of drugs directly on isolated dopaminergic neurones. The preliminary results to date suggest that the electrophysiological profile of these isolated neurones, their response to drugs, and transmitter release control mechanisms are quite similar to the mechanisms in the CNS *in situ*. For example haloperidol causes an increase in DOPAC in these isolated neurones (Seiger *et al.*, 1976; Wuerthele *et al.*, 1984). These data and the results of Trulsson (1985) support some of the conclusions drawn in this study, but many basic questions remain unresolved.

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