Exp Brain Res (1988) 69: 623–634

Experimental Brain Research

Dopamine function in the prefrontal cortex of the rat is sensitive to a reduction of tonic GABA-mediated inhibition in the thalamic mediodorsal nucleus

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Summary. Dopamine (DA) utilisation has been determined in the medial bank of the prefrontal cortex (FCx) and the agranular insular cortex (AgCx) of the rat in response to a unilateral reduction of γ-aminobutyric acid (GABA)-mediated inhibition in the thalamic mediodorsal nucleus (MD). The ratios of 3,4-dihydroxyphenylacetic acid (DOPAC) : DA and 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA): DA were used as indices of DA utilisation. A bilateral increase in both ratios was found in FCx and AgCx following unilateral infusion of GABA antagonists (1 mM) into MDc. When this concentration was infused into one MD_L no change was detected in DA utilisation of FCx, although a bilateral increase was observed in AgCx. However, a correspondence with the known anatomical connections was attained following infusion of *lower* concentrations (0.5 mM) into MD_L in that a significant bilateral elevation of DA utilisation was shown in FCx. The changes induced in these ratios by the above treatments were, in general, due to *increases* in the concentration of metabolite and slight *decreases* in that of DA. However, unilateral lesions to the presumed GABA-containing neurones of the rostrodorsal thalamic reticular nucleus (TRNd), which topographically innervate MD_L, produced increases in both metabolite *and* DA concentrations in FCx of both hemispheres, whilst those in AgCx were unaffected. Despite the slightly different results obtained using these two experimental approaches, it is argued that a reduction of tonic GABA-mediated inhibition in MD may tend to activate the DA system in cortical target regions.

Key words: Prefrontal cortex – Mediodorsal thalamic nucleus – Thalamic reticular nucleus – Dopamine utilisation – GABA antagonists

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Introduction

Midbrain dopamine (DA) neurones innervate several forebrain structures (Ungerstedt 1971; Lindvall et al. 1978) including the medial bank of the frontal cortex (FCx) and the agranular insular cortex (AgCx), which together comprise the prefrontal cortex proper (see Van Eden and Uylings 1985). Cortical DA terminal regions are also target zones for efferent neurones of the thalamic mediodorsal nucleus (MD) such that lateral portions of MD (MD_L) densely innervate FCx, whereas central portions (MDc) project to AgCx (Leonard 1969; Beckstead 1976; Krettek and Price 1977).

Recent evidence suggests that subcortical DA terminal regions are subject to profound functional control by the thalamus in both the rat and cat (Chéramy et al. 1983, 1984; Romo et al. 1983; Kilpatrick and Phillipson 1986; Kilpatrick et al. 1986a, c). However, less attention has been paid to possible thalamic control of *cortical* DA systems. The present study, therefore, set out to examine the role of MD in regulating DA function in cortical target areas. Since most MD neurones have a low spontaneous firing rate (Vives and Mogenson 1985), experimental lesions of MD might, at best, have only a minor effect on cortical DA function. In contrast, although application of an electrical stimulus or an excitotoxic compound to MD appeared to increase cortical DA utilisation (Jones et al. 1985; 1986a, 1987), these procedures will inevitably cause slight tissue damage. Moreover, the former may also concurrently excite fibres of passage and antidromically activate MD afferents. Consequently, a different approach has been adopted here.

An attempt has been made in the present study to modify MD neuronal activity *indirectly* by manipulating a major afferent route to MD from the thalamic reticular nucleus (TRN). Efferent neurones from

TRN project extensively and exclusively to the thalamus (Scheibel and Scheibel 1966; Minderhoud 1971; Jones 1975; Ohara and Lieberman 1985) and, in particular, MD_L has been shown to receive a very dense and topographic input from rostro-dorsal TRN (TRNd) (Cornwall and Phillipson 1987; see also Jones 1975; Siegel et al. 1977; Rebollo 1985). This connection between TRN and thalamus has been shown to be at least partly inhibitory (Schlag and Waszak 1970; Yingling and Skinner 1975; Skinner and Yingling 1976; Mushiake et al. 1984; Steriade and Deschênes 1984; Steriade et al. 1985; Mulle et al. 1986) and the majority of TRN neurones are GABA-containing (Houser et al. 1980; Oertel et al. 1983; Benfey et al. 1985; de Biasi et al. 1986) and form presumed inhibitory synapses on thalamic target cells (Ohara et al. 1980; Montero and Scott 1981). Thus, the present paper has investigated the functional effects of an overall decrease in GABA-mediated inhibition in MD on cortical DA transmission produced (a) acutely, by direct injection of GABA antagonists into MD or (b) chronically, via a cell-specific lesion of TRNd. Some of these results have been communicated in preliminary form (Cornwall et al. 1986).

Methods

Surgical procedure

Male Porton rats (150–180 g, Wistar-derived) were anaesthetized using halothane (1.5% in O_2) and secured in a Kopf stereotaxic frame. A midline incision (10 mm) was made through the skin overlying the skull and the scalp reflected. A burr hole was drilled on one side of the skull to allow unilateral penetration of a 1 µl Hamilton syringe (0.48 mm o.d.) to the appropriate co-ordinate for locating either MD_L (A –2.15 from bregma; L 0.8; V 5.0 from cortical surface), MDc (A –2.15; L 0.55; V 5.0) or TRNd (A –0.9; L 2.1; V 5.45).

Bicuculline (Bic) and picrotoxin (Pic) injections

A mixture of bicuculline and picrotoxin (Bic/Pic) was prepared for injection into MD. The injection volume of 0.2 µl contained either (i) 46 ng (–)-bicuculline methobromide and 60 ng picrotoxin (Sigma, St. Louis, USA), final concentration 1.0 mM or (ii) 23 ng Bic and 30 ng Pic, final concentration 0.5 mM. Both solutions were made up in Krebs bicarbonate buffer gassed with 95% O_2/5% CO_2. The injection was placed into MD_L or MDc and, although the penetrations were unilateral, they were performed into either the left or right hemisphere. The Bic/Pic mixture was delivered stepwise at 20 s intervals for 3 min, after which the needle was slowly withdrawn and the wound cleaned with 10% povidone-iodine USP (Betadine®, Napps Labs. Ltd.) and sprayed with the antibiotics neomycin, polymyxin and bacitracin (Tribiotic®, Riker Labs.). Finally, the skin was closed with a suture clip and the animals allowed to recover for 15 min. Sham-operated animals received an injection of Krebs bicarbonate buffer into either MD_L or MDc.

Ibotenic acid injection

The excitotoxin sodium ibotenate (IBO) was used to lesion TRNd efferent neurones since it has repeatedly been shown that its actions spare axons of passage and do not appear to cause distant lesions (Guldin and Markowitsch 1981; Köhler and Schwarcz 1983). Ibotenic acid was first dissolved in 0.1 M NaOH and diluted with 0.15 M NaCl to produce a 12.5 mM solution at pH 7.1. Injection of IBO was performed stepwise at 20 s intervals for a period of 3 min (2 µg in 0.2 µl). The syringe remained *in situ* for a further 1 min before being slowly withdrawn. In sham-operated animals the IBO-filled Hamilton syringe was lowered only as far as the hippocampus (V 3.8) without an injection being made. Finally, the wound was carefully cleaned and sealed as above. A recovery period of 2 days was used to ensure the development of a histologically verifiable lesion.

Neurochemical analysis: dissection and sample preparation

After the appropriate recovery period the animals were killed by cervical fracture and their brains removed onto an ice-cold Petri-dish soaked with chilled Krebs buffer. Using a chilled razor blade, coronal sections were made through the cortex. From these slices FCx and AgCx were rapidly but carefully dissected (see Jones et al. 1986b, Fig. 1), and immediately frozen on a dry-ice cooled plate of aluminium. The frozen tissue blocks were then thawed, blotted and rapidly weighed on a torsion balance. Subsequent homogenisation of these blocks in a 1.8 ml polypropylene flip-top vial, containing 600 µl of ice-cold mobile phase spiked with internal standard (see below), was achieved using a tapered teflon motorised pestle. The samples were subjected to further disruption by freezing at –70° C and when required the homogenates were thawed and centrifuged using an IEC Centra-3RS refrigerated centrifuge (15,000 g, 20 min, 4° C). The extracted supernatant was filtered (Acro-LC 13, 0.45 µm Gelman Sciences, Northampton, U.K.) and 100 µl of the filtrate was then assayed for DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (Homovanillic acid, HVA) by reverse-phase high performance liquid chromatography (HPLC) with coulometric detection as previously described (Kilpatrick et al. 1986b). Standard solutions containing known amounts of analyte (0.3–10 ng ml^{-1}) and standard (5 ng ml^{-1}) were frozen and then run in parallel with the samples.

Chromatographic hardware and conditions

This is described in detail elsewhere (Kilpatrick et al. 1986b). Briefly, the chromatographic column was an Ultratechsphere (C_{18}, 5 µm, 250 × 4.6 mm) and an ESA Coulochem 5100 A controller was used to apply potentials of +450 mV to a 5020 Guard cell and –0 mV and +340 mV to detectors 1 and 2 respectively of a model 5011 analytical cell. A Gilson 303 pump was fitted along with a Gilson 802 manometric module with pulse dampener and a Rheodyne 7125 injection valve. The mobile phase (pH 4.35) consisted of 0.1 M sodium acetate, 0.05 M citric acid, 130 µM disodium EDTA, 230 µM 1-octanesulphonic acid (SOS) and 11% methanol in Milli-Q® water. The mobile phase was filtered then sparged with helium before being pumped through the system at 1.0 ml min^{-1}.

Histology

The block of brain tissue remaining after the dissection was placed in 10% v/v formal-saline for one week. Sections were cut on a Leitz

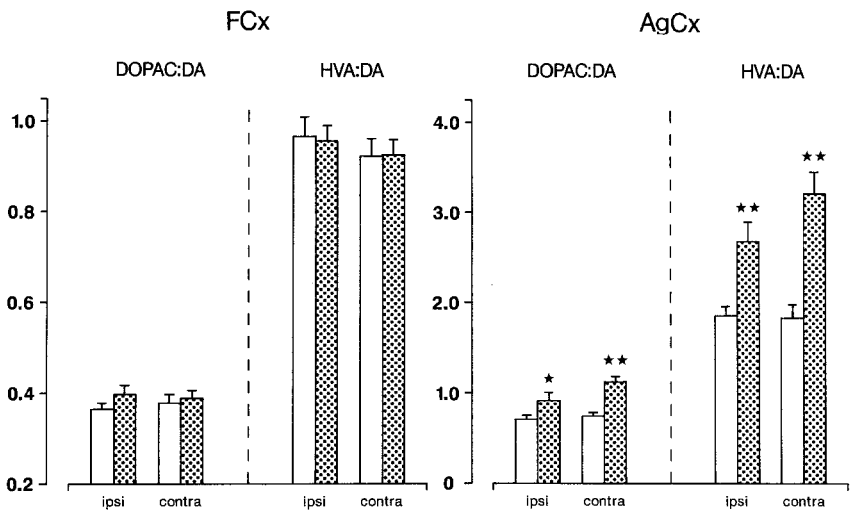

Fig. 1. DA utilization in FCx and AgCx 15 min after unilateral infusion of GABA antagonists into MD_L of either left or right hemisphere. The ratios of DOPAC : DA and HVA : DA are shown ipsilateral (ipsi) and contralateral (contra) to the infusion. Values represent the mean ± SEM. Control data is taken from 21–23 rats for FCx and from 16 rats for AgCx, whilst Bic/Pic data is the mean value taken from 12–13 rats. * $p < 0.05$; ** $p < 0.005$. Open columns, sham control; shaded columns, Bic/Pic (1 mM in 0.2 µl) infusion

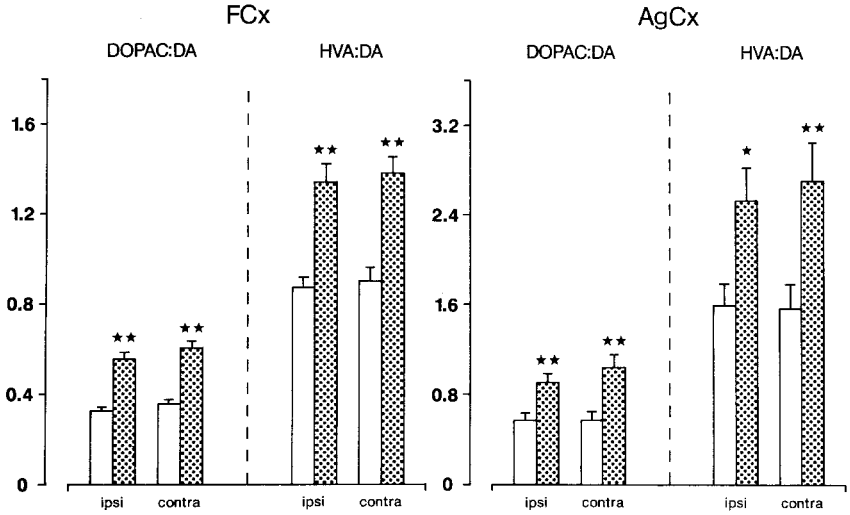

Fig. 2. DA utilisation in FCx and AgCx 15 min after unilateral infusion of GABA antagonists into MDc of either left or right hemisphere. The ratios of DOPAC : DA and HVA : DA are shown ipsilateral (ipsi) and contralateral (contra) to the infusion. Values represent the mean ± SEM of data from 9–10 rats. * $p < 0.025$; ** $p < 0.005$. Open columns, sham control; shaded columns, Bic/Pic (1 mM in 0.2 µl) infusion

freezing microtome at 40 µm and were stained with thionin to determine the site of injection or lesion. These sites were charted onto planes adapted from the atlas of König and Klippel (1963).

Chemicals

Only HPLC or analytical grade chemicals were employed. Citric acid, anhydrous sodium acetate and disodium EDTA were from Fluka, Buchs, Switzerland; methanol from Rathburn Chemicals Ltd., Walkerburn, Scotland; SOS from Kodak Eastman, Liverpool; ibotenic acid from Serva; and all other chemicals from Sigma, Poole, U.K.

Data analysis

The data was analysed using either a Bryans chart recorder or a Spectra Physics SP 4270 integrator which was programmed to compute a peak area ratio of internal standard to analyte. Results were finally expressed as ng mg^{-1} wet weight of tissue. Statistical analysis was performed using the Mann-Whitney U-test.

Results

Injections of Bic/Pic

1 mM Bic/Pic (Figs. 1 and 2; Tables 1 and 2). The effects on cortical DA utilisation of injections of Bic/Pic into MD of either the right or left hemisphere were not dependent upon the side of injection. Consequently, data from right and left side injections were combined. Injections of Bic/Pic centred on MD_L (Fig. 1) had no effect on DA utilisation in FCx. However, both DOPAC : DA and HVA : DA increased in the ipsilateral AgCx (+29%, $p < 0.05$; +45%, $p < 0.005$, respectively) and slightly greater increases were observed contralaterally (+53%, $p < 0.005$; +75%, $p < 0.005$, respectively). The enhanced ratios in ipsilateral AgCx occurred as a result of significant increases in the tissue content of

Table 1. The tissue concentration (ng mg^{-1} wet wt.) of DOPAC, HVA and DA 15 min after unilateral infusion of GABA antagonists (Bic/Pic, 1 mM in 0.2 µl) into MD_L of either left or right hemisphere. The concentrations were determined in ipsilateral (IPSI) and contralateral (CONTRA) FCx and AgCx. Values represent the mean ± SEM and the number of animals are shown in parentheses

Hemisphere	Cortical region	Treatment	Concentration (ng mg^{-1}) DOPAC	HVA	DA
IPSI	FCx	Control	0.052 ± 0.003 (23)	0.140 ± 0.011 (23)	0.144 ± 0.008 (23)
		Bic/Pic	0.055 ± 0.003 (13)	0.132 ± 0.011 (12)	0.140 ± 0.011 (13)
CONTRA	FCx	Control	0.051 ± 0.003 (21)	0.127 ± 0.009 (21)	0.140 ± 0.010 (21)
		Bic/Pic	0.052 ± 0.003 (13)	0.124 ± 0.007 (13)	0.135 ± 0.008 (13)
IPSI	AgCx	Control	0.049 ± 0.002 (16)	0.125 ± 0.006 (16)	0.070 ± 0.005 (16)
		Bic/Pic	*0.056 ± 0.004 (13)	***0.161 ± 0.010 (13)	0.063 ± 0.004 (12)
CONTRA	AgCx	Control	0.042 ± 0.003 (16)	0.103 ± 0.007 (16)	0.057 ± 0.003 (16)
		Bic/Pic	**0.052 ± 0.003 (12)	***0.148 ± 0.015 (12)	***0.045 ± 0.002 (12)

* $p < 0.05$; ** $p < 0.025$; *** $p < 0.005$

Table 2. The tissue concentration (ng mg^{-1} wet wt.) of DOPAC, HVA and DA 15 min after unilateral infusion of GABA antagonists (Bic/Pic, 1 mM in 0.2 µl) into MDc of either left or right hemisphere. The concentrations were determined in ipsilateral (IPSI) and contralateral (CONTRA) FCx and AgCx. Values represent the mean ± SEM and the number of animals are shown in parentheses

Hemisphere	Cortical region	Treatment	Concentration (ng mg^{-1}) DOPAC	HVA	DA
IPSI	FCx	Control	0.050 ± 0.003 (10)	0.134 ± 0.010 (10)	0.153 ± 0.007 (10)
		Bic/Pic	***0.069 ± 0.003 (10)	**0.167 ± 0.010 (10)	***0.126 ± 0.006 (10)
CONTRA	FCx	Control	0.046 ± 0.003 (10)	0.115 ± 0.008 (10)	0.128 ± 0.007 (10)
		Bic/Pic	***0.062 ± 0.003 (10)	**0.144 ± 0.007 (10)	*0.111 ± 0.011 (10)
IPSI	AgCx	Control	0.047 ± 0.006 (10)	0.129 ± 0.015 (10)	0.085 ± 0.008 (10)
		Bic/Pic	0.054 ± 0.002 (9)	*0.149 ± 0.010 (9)	*0.063 ± 0.006 (9)
CONTRA	AgCx	Control	0.039 ± 0.004 (10)	0.106 ± 0.009 (10)	0.077 ± 0.010 (10)
		Bic/Pic	*0.050 ± 0.004 (9)	*0.128 ± 0.008 (9)	**0.051 ± 0.005 (9)

* $p < 0.05$; ** $p < 0.025$; *** $p < 0.005$

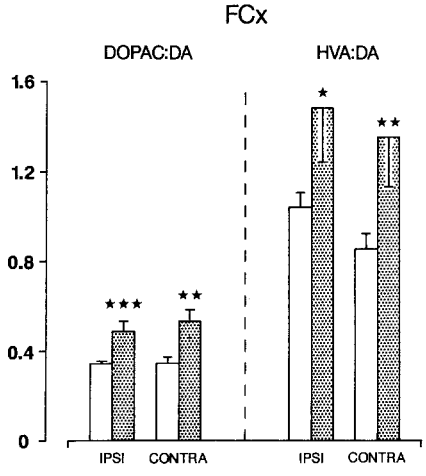

Fig. 3. DA utilisation in FCx 15 min after unilateral infusion of GABA antagonists into MD_L of right hemisphere. The ratios of DOPAC : DA and HVA : DA are shown ipsilateral (IPSI) and contralateral (CONTRA) to the infusion. Values represent the mean ± SEM of data from 5–8 rats. * $p < 0.05$; ** $p < 0.025$; *** $p < 0.005$. Open columns, sham control; shaded columns, Bic/Pic (0.5 mM in 0.2 µl) infusion

individual metabolites (DOPAC, +16%, $p < 0.05$; HVA, +28%, $p < 0.005$), whereas the concentration of DA was not significantly different from controls (Table 1). In the contralateral hemisphere, however, the enhanced ratios occurred as a result of both an increase in the tissue content of DOPAC and HVA (+24%, $p < 0.025$; +44%, $p < 0.005$, respectively) and a decrease in that of DA (–22%, $p < 0.005$). This significant decline in DA content accounts in part for the more marked increases in DA utilisation ratios observed in the contralateral compared with the ipsilateral hemisphere.

In contrast with the results obtained following intra-MD_L infusion of Bic/Pic, injections of these GABA antagonists into MDc (Fig. 2) produced bilaterally symmetrical increases in FCx of both DOPAC : DA (ipsilateral: +70%, $p < 0.005$; contralateral: +68%, $p < 0.005$) and HVA : DA (ipsilateral: +54%, $p < 0.005$; contralateral: +53%, $p < 0.005$). The content of individual metabolites (Table 2) increased ipsilaterally (DOPAC +37%, p

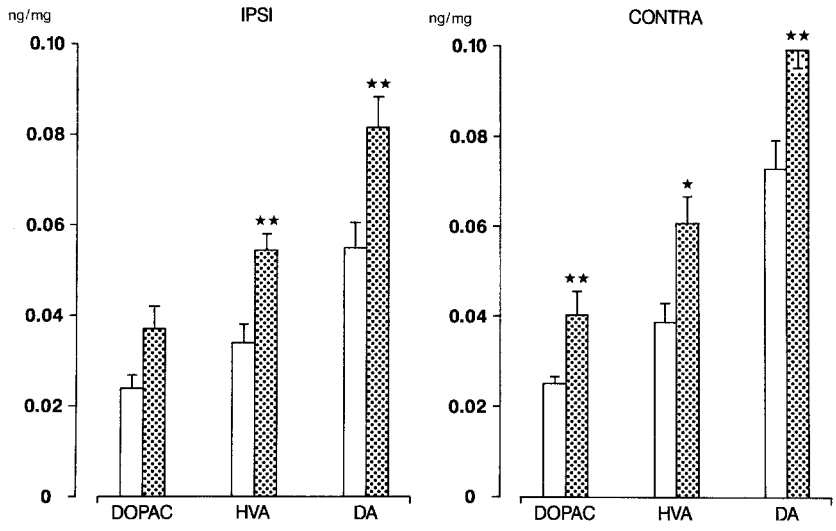

Fig. 4. The tissue concentration (ng mg^{-1} wet wt.) of DOPAC, HVA and DA in FCx 2 days after unilateral infusion of ibotenate into TRNd. The concentrations were determined in both ipsilateral (IPSI) and contralateral (CONTRA) FCx. Values represent the mean ± SEM of data from 4–6 rats. * $p < 0.025$; ** $p < 0.01$. Open columns, sham control; shaded columns, ibotenate infusion (2 μg in 0.2 μl)

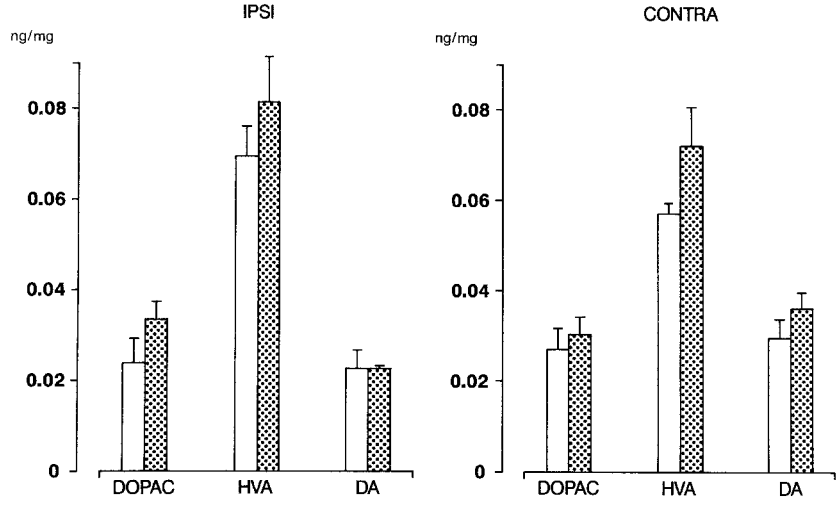

Fig. 5. The tissue concentration (ng mg^{-1} wet wt.) of DOPAC, HVA and DA in AgCx 2 days after unilateral infusion of ibotenate into TRNd. The concentrations were determined in both ipsilateral (IPSI) and contralateral (CONTRA) AgCx. Values represent the mean ± SEM of data from 5–6 rats. Open columns, sham control; shaded columns, ibotenate infusion (2 μg in 0.2 μl)

< 0.005; HVA +25%, $p < 0.025$) and contralaterally (DOPAC +35%, $p < 0.005$; HVA +25%, $p < 0.025$), whilst a significant decrement in DA content was observed in both hemispheres (ipsilateral: –18%, $p < 0.005$; contralateral: –14%, $p < 0.05$). In AgCx (Fig. 2), the increases in ipsilateral DOPAC : DA (+58%, $p < 0.005$) and HVA : DA (+59%, $p < 0.025$) were slightly less marked than those obtained in the contralateral hemisphere (+83%, $p < 0.005$; +73%, $p < 0.005$, respectively). These changes in DA utilisation ratios occur as a result of increases in the tissue content (Table 2) of DOPAC (contralateral: +29%, $p < 0.05$) and HVA (ipsilateral: +16%, $p < 0.05$; contralateral: +21%, $p < 0.05$), together with significant declines in the content of DA (ipsilateral: –25%, $p < 0.05$; contralateral: –34%, $p < 0.025$).

0.5 mM Bic/Pic (Fig. 3). Significant increases in DA utilisation in FCx were detected ipsilaterally (DOPAC : DA +42%, $p < 0.005$; HVA : DA +43%, $p < 0.05$) and contralaterally (DOPAC : DA +53%, $p < 0.025$; HVA : DA +58%, $p < 0.025$) in response to an injection into MD_L of the right hemisphere. Although the tissue contents of individual metabolite and parent amine were not significantly different from controls, in each case there was a trend towards in increase in metabolite content and a decrease in that of DA.

TRN lesion: 2 day recovery (Figs. 4 and 5)

Infusion of IBO into TRNd failed to significantly alter DA utilisation ratios in either FCx or AgCx.

However, in FCx significant increases were detected (Fig. 4) in the content of DOPAC (contralateral: +62%, $p < 0.01$), HVA (ipsilateral: +60%, $p < 0.01$; contralateral: +57%, $p < 0.025$) and DA (ipsilateral: +49%, $p < 0.01$; contralateral: +36%, $p < 0.01$). No significant effects were observed on the tissue content of either metabolite or parent amine in AgCx, although HVA levels tended to be slightly greater than controls (Fig. 5).

Histology

TRN lesion (Figs. 6 and 7a, b). Cell loss and glial infiltration had occurred 2 days after an injection of IBO into TRN. The injection was centred on TRNd, although some spread occurred into surrounding regions, i.e. anteromedial (AM), anteroventral (AV), laterodorsal (LD) and ventrolateral (VL) thalamic nuclei.

Bic-Pic injections (Fig. 8a, b). Relatively little tissue damage is caused by injections of Bic/Pic (1 mM) into either MD_L (Fig. 8a) or MDc (Fig. 8b).

Discussion

The ratios of DOPAC : DA and HVA : DA are thought to reflect changes in metabolism and/or release, although HVA : DA may more adequately reflect synaptic DA release (see Commissiong 1985; Kilpatrick et al. 1985; Kilpatrick and Phillipson 1986). Nevertheless, increases obtained here in DOPAC : DA generally parallel those of HVA : DA. In control animals these indices of DA utilisation were greater in AgCx compared with FCx confirming data obtained previously in animals not exposed to anaesthesia (Jones et al. 1986b).

The present experiments show that a reduction of GABA-mediated inhibition within MD, generated either by a cell-specific lesion of the presumed GABA-containing projections from TRNd or by the

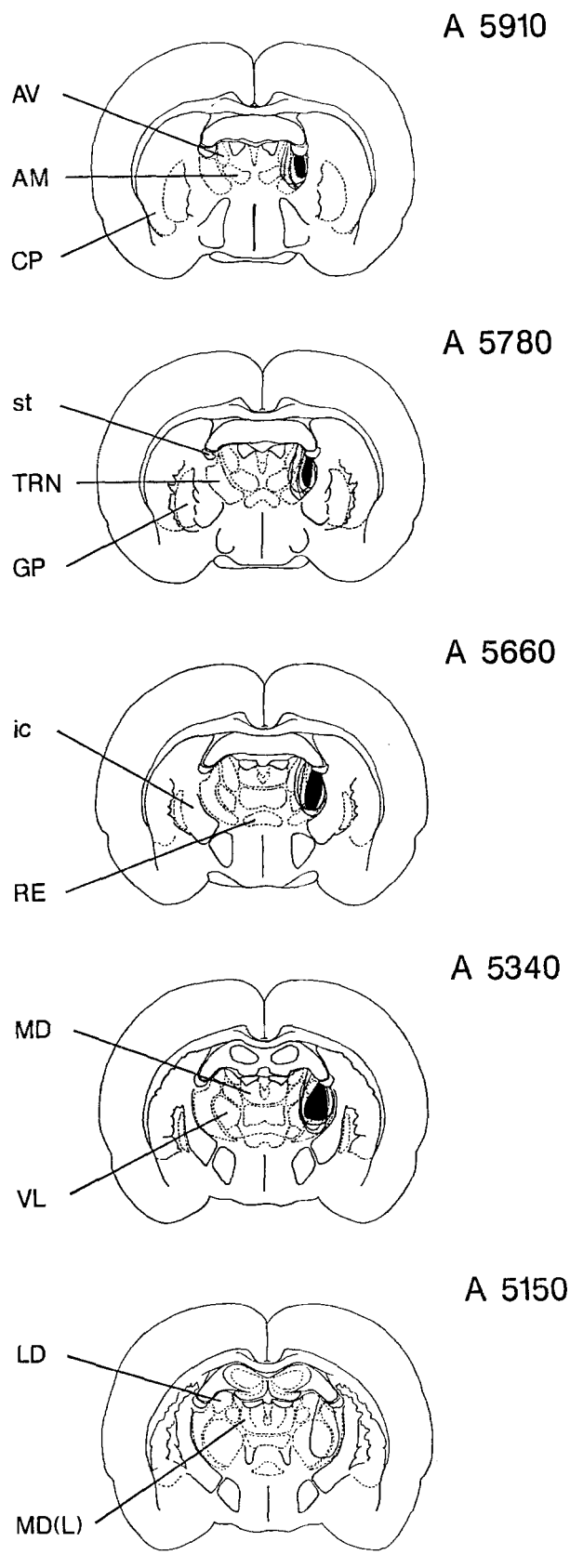

Fig. 6. Rostro-caudal series of sections taken through the rat thalamus and charted onto planes adapted from König and Klippel (1963). Each section represents a composite reconstruction of the extent of ibotenate infusion after a recovery period of 2 days. Filled areas in individual sections correspond to areas of overlap between two or more of these lesion sites. Abbreviations: AM, anteromedial thalamic nucleus; AV, anteroventral thalamic nucleus; CP, caudate putamen; GP, globus pallidus; ic, internal capsule; LD, laterodorsal thalamic nucleus; MD/MD(L), mediodorsal thalamic nucleus/lateral division; RE, reuniens thalamic nucleus; VL, ventrolateral thalamic nucleus

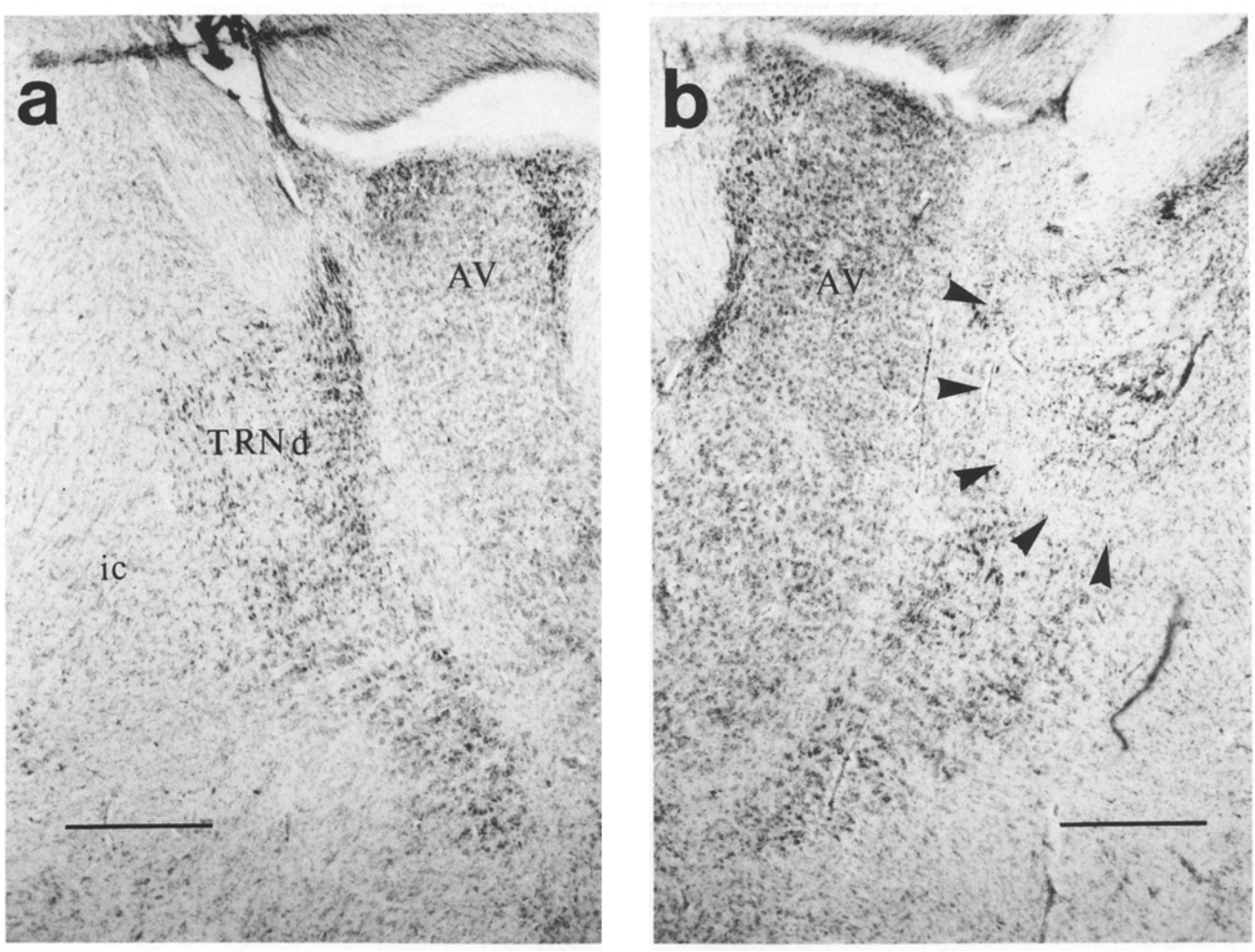

Fig. 7. a Low power view of TRN and surrounding structures taken from the hemisphere opposite to the side of injection. TRNd, thalamic reticular nucleus, dorsal portion; AV and ic, see Fig. 6 legend. Bar = 500 μm. **b** Low power view of TRN and surrounding structures taken from the hemisphere injected with IBO. Arrowheads indicate the margin of the lesion. Bar = 500 μm

injection of GABA antagonists directly into MD, produces alterations in cortical DA function. However, the changes induced by these different experimental protocols were not identical since infusion of GABA antagonists into MD increased cortical DOPAC : DA and HVA : DA ratios, whereas these values were unaffected by lesions of TRNd (for discussion, see below).

Bicuculline/Picrotoxin (Bic/Pic) injections

Clearly, acute injection of GABA antagonists into MD produces an increase in cortical DA utilisation. However, the pattern of changes do not always correlate with the known segment-specific anatomy. Thus, projections from MD_L predominantly innervate FCx, whereas AgCx is mainly innervated by neurones located in MDc (Leonard 1969; Beckstead 1976; Krettek and Price 1977). Despite this, infusion of Bic/Pic (1 mM) into MD_L had, surprisingly, no effect on either DA or metabolite content in FCx. DA utilisation was, however, markedly elevated in AgCx of both hemispheres. Moreover, infusion of the same concentration of Bic/Pic into MDc produced a bilateral increase in DA utilisation not only in AgCx but also in FCx. These apparently discrepant findings are probably the result of a concentration dependent phenomenon, since lower concentrations of Bic/Pic (0.5 mM) produced significant bilateral increases in DA utilisation in FCx following infusion into MD_L; effects which are in accordance with the known anatomical connections.

Both electrical stimulation and short-term excitotoxin treatment of MD_L have also been shown to enhance DA utilisation in FCx, whilst lesions of MD_L neurones have little effect (Jones et al. 1985, 1986a, 1987). Thus, lower concentrations of Bic/Pic

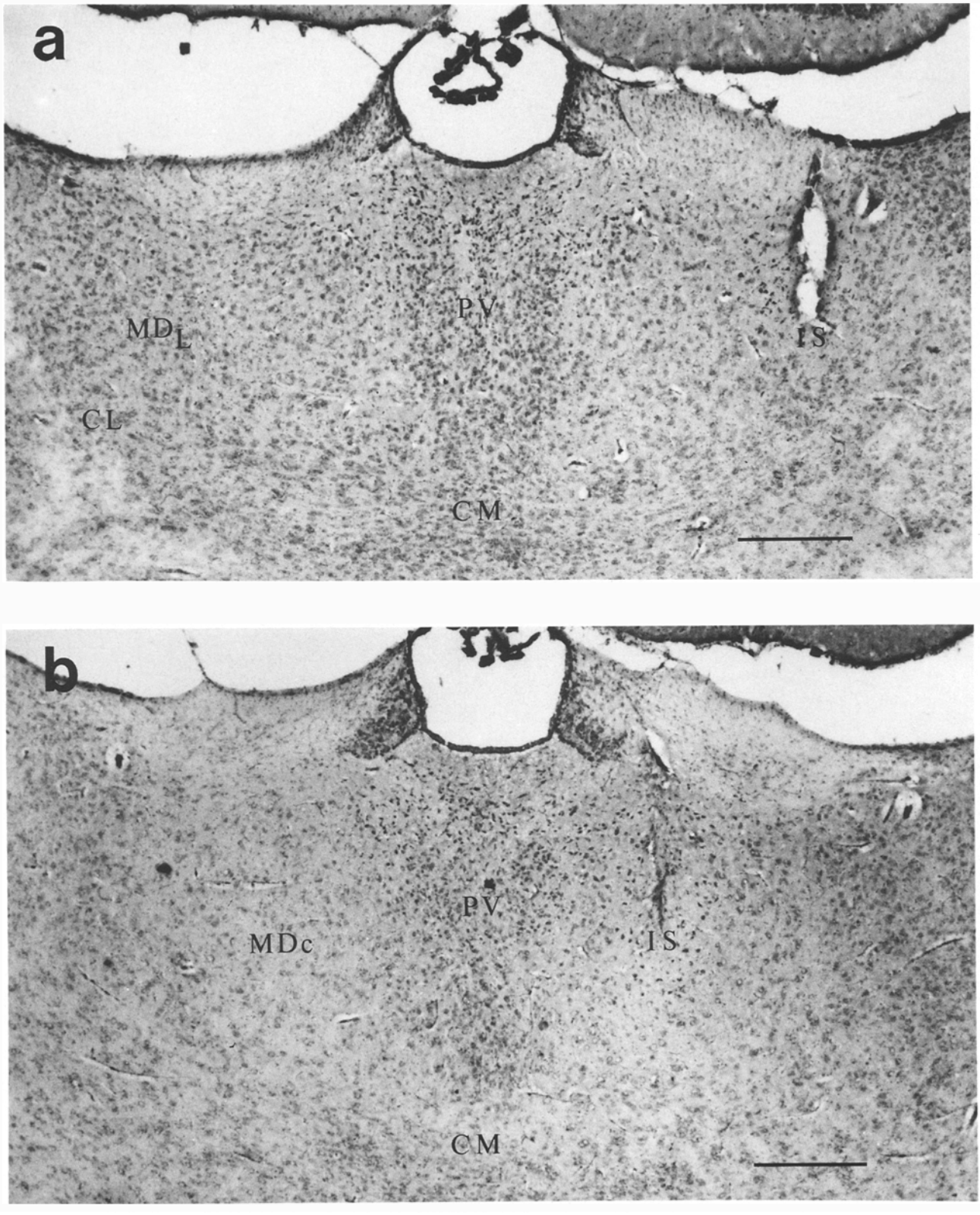

Fig. 8. a Low power view of MD showing the needle tract and injection site (IS) after infusion of Bic/Pic (1 mM) into MD_L. Abbreviations: CL, centrolateral thalamic nucleus; CM, central medial thalamic nucleus; MDc and MD_L mediodorsal thalamic nucleus, central and lateral divisions, respectively; PV, paraventricular thalamic nucleus. Bar = 400 μm. **b** Low power view of MD showing the needle tract and injection site (IS) after infusion of Bic/Pic (1 mM) into MDc. Bar = 400 μm

may effectively *excite* MD_L neurones. Indeed, a previous study has shown that the firing rate of MD cells increases in response to iontophoretically applied picrotoxin (Vives and Mogenson 1985). However, higher concentrations may actually *depress* activity in these neurones, possibly as a result of over-stimulation. The enhanced DA utilisation seen in *AgCx* following infusion into *MDc* of the higher Bic/Pic concentration may be explained by a greater degree of tonic GABA-mediated inhibition in MDc compared with MD_L. In this respect, it may be pertinent that the greatest accumulation of GAD immunoreactivity in MD occurs in its central division (Zahm et al. 1987). However, effective blockade of GABA-mediated inhibition was attempted in the present study by combining Pic, an antagonist at the chloride ionophore (Ticku et al. 1978) with Bic, a competitive antagonist at the GABA A site (Enna et al. 1977; Möhler and Okada 1977), since the efficacy of Pic is less likely to be compromised under conditions of high tonic GABA release.

The effects in AgCx and FCx following infusion into MD_L and MDc respectively may be due to some spread of drug between adjacent segments and/or to connections between these two cortical areas. For example, efferents from FCx project to both ipsilateral and contralateral AgCx, although the reciprocal projections are only very weak (Beckstead 1979; Brutus et al. 1984). In addition, there are a small number of neurones located close to the medial border of MDc which send axon collaterals to both FCx and AgCx (Sarter and Markowitsch 1984).

The low tonic activity of most MD neurones (Vives and Mogenson 1985) may be due to either an inherent neuronal characteristic or to profound synaptic inhibition from input neurones. Retrograde tracing studies in our laboratory (Cornwall and Phillipson 1987) have shown that MD receives input from several GABA-containing regions (e.g. substantia nigra, ventral pallidum and TRN). The projections from nigra to several thalamic nuclei appear to contain GABA (Kilpatrick et al. 1980; Starr and Kilpatrick 1981; Gerfen et al. 1982), although the transmitter used in nigral projections to MD is not known. Furthermore, the majority of ventral pallidal projections to MD probably use a transmitter other than GABA (Vives and Mogenson 1985; Zahm et al. 1987). However, in terms of input density, the projections from TRN to MD appear to be more dominant than those from either of these other GABA-rich areas. These findings, together with the fact that Ottersen and Storm-Mathisen (1984) failed to find a TRN cell which did *not* show GABA-like immunoreactivity, suggests that the effects of Bic/Pic observed here after infusion into MD_L are, perhaps, predominantly due to antagonism of GABA following its release from TRN neurones.

In summary, infusion of GABA antagonists into MD produced an increase in cortical DA utilisation consistent with findings obtained previously using electrical stimulation and short-term infusion of an excitant amino acid (Jones et al. 1985, 1986a, 1987). In contrast with these previous studies, however, the increased DA utilisation obtained here following intra-thalamic Bic/Pic infusion was partly due to a decline in DA itself (see below)

Habenular influences

Since diffusion of Bic/Pic from the injection centre could not be determined accurately, the possibility remains that some of the drug reached surrounding structures. For example, the habenula (HB) projects to DA cell body regions (Herkenham and Nauta 1979; Phillipson 1978) and appears to exert a tonic inhibitory influence over DA neurones (Lisoprawski et al. 1980; Christoph et al. 1986; Nishikawa et al. 1986). Since GABA is released from afferents to HB which travel in the stria medullaris (Gottesfeld and Jacobowitz 1978; Gottesfeld et al. 1980), dorsal spread of Bic/Pic into HB is likely to antagonise the actions of GABA released from these neurones. If this were the case, however, then cortical DA utilisation would be expected to *decrease* in response to an injection of Bic/Pic. Thus, the present *increase* in cortical DA utilisation, together with the fact that MD manipulations never appear to affect cell body DA function (unpublished work), suggests that the effects of Bic/Pic are probably mediated via MD rather than HB.

Lesion of the thalamic reticular nucleus

DA utilisation ratios were unaffected by a 2 day old lesion of TRNd. However, the tissue content of DOPAC, HVA and DA in FCx were all markedly and bilaterally elevated by this treatment. These results differ from those obtained following an acute infusion of GABA antagonists into MD_L in that the latter treatment not only elevated DA utilisation ratios but did so by increasing the cortical content of metabolite and *decreasing* that of DA. One possible explanation for these findings is that DA synthesis may have increased over and above its rate of utilisation 2 days after a lesion of TRNd, whereas the rate of DA synthesis 15 min following injections of Bic/Pic may not have been sufficient to replenish DA following its enhanced utilisation. Alternatively, Bic/

Pic may additionally antagonise the responses to GABA following its release from MD_L afferents other than those originating in TRN. Other factors, such as exposure of animals undergoing acute injection of GABA antagonists to the effects of residual anaesthetic may also be pertinent. Indeed, halothane has been shown to influence DA release and metabolism in both the rat (Ford and Marsden 1986; Kilpatrick et al. 1985; Kilpatrick and Phillipson 1986) and cat (Nieoullon et al. 1977) and halothane anaesthesia also seems to increase metabolic activity in TRN (Peschanski et al. 1986).

The effects observed on DA function in FCx after a TRNd lesion are probably mediated, at least in part, via MD_L rather than by a direct route from TRNd to cortex since it is reported that there are no corticopetal projections from TRN (Jones 1975). The lesion was centered on TRNd although there was some unavoidable spread to AM, AV, LD and VL. Since the cortical projection areas of these nuclei differ from those of MD, with the possible exception of AM (Domesick 1972; Krettek and Price 1977; Finch et al. 1984), it appears likely that the effects produced on the DA system in FCx were due to lesions of TRN neurones rather than those of neighbouring nuclei.

Anatomical substrates

It has previously been suggested that changes induced in cortical DA utilisation following manipulation of MD neurones occur at the level of the DA terminal (Jones et al. 1987). Indeed, the terminal innervation from MD overlaps that from midbrain DA neurones in that MD neurones terminate chiefly in layers I, III and VI of both cortical zones (Leonard 1969; Domesick 1972; Jacobson and Trojanowski 1975; Krettek and Price 1977), whilst midbrain DA neurones terminate in all layers but predominantly in layers V and VI (Berger et al. 1976; Lindvall et al. 1978; Van Eden et al. 1987). However, the presence of direct axo-axonic contact between MD efferent neurones and DA fibres has yet to be shown and a knowledge of the circuitry must therefore await detailed morphological characterisation of cortical synaptology.

Bilaterality

Both intra-MD Bic/Pic injections and TRNd lesions were made unilaterally yet they produced bilateral changes in cortical DA function. This is curious since MD projections to cortex are strictly unilateral (Krettek and Price 1977) but may be explained by the fact that both FCx and AgCx project homotopically to the opposite hemisphere (Beckstead, 1979; Sarter and Markowitsch 1985). Interhemispheric connections between the thalami are unlikely to give rise to these bilateral changes following unilateral TRNd lesions since the input from TRN to thalamic nuclei appears to be unilateral in the rat (Ohara and Lieberman 1985).

Summary

Despite some subtle differences, the increases in cortical DA utilisation seen here following an injection of Bic/Pic into MD compare favourably with previous studies using other methods to stimulate MD neurones directly (Jones et al. 1985, 1986a, 1987). Furthermore, other thalamic nuclei appear to enhance *subcortical* DA utilisation/release when stimulated (cat: McLennan 1964; rat: Kilpatrick et al. 1985; Kilpatrick and Phillipson 1986). Reduction of GABA-mediated inhibition in MD was also attempted by lesioning TRNd and, whilst the indices of DA utilisation were not affected by this treatment, the cortical content of DA and its metabolites were increased. These findings, together with those of previous studies which show that *lesions* of thalamic nuclei, if anything, slightly *decrease* forebrain DA utilisation (Kilpatrick et al. 1986a; Jones et al. 1987), support the general contention that enhanced neuronal activity in output fibres from thalamic nuclei tend to increase DA release in their respective convergent terminal regions.

Acknowledgements. The authors gratefully acknowledge the support of the Wellcome Trust. We also thank Miss Sheila Travers for typing the manuscript, and Mr. P. Darby and Mr. A.C. Griffiths for the photography.

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Received April 22, 1987 / Accepted August 5, 1987