J. Pharm. Pharmac., 1978, 30, 158–161 Received July 28, 1977

Circling behaviour in the rat following unilateral injections of *p*-chlorophenylalanine and ethanolamine-*O*-sulphate into the substantia nigra

TREVOR TANNER

Applied Pharmacology Laboratories, Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cardiff CF1 3NU, U.K.

Circling behaviour following the unilateral intranigral injection of the γ-aminobutyric acid (GABA) transaminase inhibitor ethanolamine-*O*-sulphate, (EOS), or the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (pCPA) was compared, both for spontaneous circling and for circling induced by apomorphine administration. EOS-injected rats exhibited spontaneous contralateral rotation for up to 3 h after such injections. pCPA-injected rats showed only a slight ipsilateral postural asymmetry, both groups of rats elicited ipsilateral rotation upon apomorphine challenge. Increased ipsilateral dopamine release within the corpus striatrum accompanied this response. It is proposed that GABA and 5-HT may be closely related in modifying the nigrostriatal impulse flow.

In rats with unilateral nigrostriatal degeneration, but bilaterally intact striatal dopamine receptors, directly acting dopamine agonists such as apomorphine induce circling behaviour away from the side of the lesion. It has become widely accepted that this is because the dennervated receptors have become supersensitive (Ungerstedt, 1971). Rats with intact nigrostriatal connections, but with unilaterally elevated nigral γ-aminobutyric acid (GABA) contents, circle towards the side of increased GABA concentrations if injected intraperitoneally with apomorphine (Dray, Fowler & others, 1975). Furthermore, if the GABA receptor agonist muscimol or the GABA analogue β-*p*-chlorophenyl GABA are injected unilaterally into one substantia nigra (SN), they produce contralateral rotation, an action presumably produced by an imbalanced GABA receptor activation and indicates that the net effect of GABA on the nigrostriatal dopaminergic pathway is excitatory (Scheel-Knüger, Arnt & Magelund, 1977; Waddington, 1977). However, biochemical, electrophysiological and pharmacological evidence indicate that inhibition of neuronal activity in the SN by the ipsilateral caudate nucleus is mediated by GABA (Feltz, 1971; Kim, Bak & others, 1971; Precht & Yoshida, 1971). Taking these reports together, it appears that GABA modulates the nigrostriatal pathway indirectly, perhaps through an inhibitory action on one or more other transmitters within the SN.

Some discussion exists about the possible role of various neurotransmitters in the SN (Dray & Straughan, 1976). 5-Hydroxytryptamine (5-HT) has both excitatory and inhibitory actions, when applied iontophoretically to SN neurons (Dray, Gonye & others, 1976). Evidence exists suggesting a 5-HT influence onto SN neurons originating from the median raphe nucleus (Hadju, Hassler & Bak, 1973; Dray & others, 1976) and contralateral rotation occurs after asymmetric medial raphe nuclei lesions in the rat (Costall & Naylor, 1974). I have, therefore, compared the response to unilaterally elevated GABA and unilaterally reduced 5-HT concentrations in the SN, on circling behaviour in the rat.

MATERIALS AND METHODS

Male al bino Wistar rats (150–200 g) were anaesthetized with halothane and unilateral injections of the GABA transaminase inhibitor ethanolamine-*O*-sulphate (EOS, 25-100 μg) or the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (pCPA; 25-100 μg dissolved in 0·9% saline containing 1% ascorbate) or saline, in an injection volume of 1 μl, were made using a 5 μl Hamilton syringe, stereotaxically placed in one SN (AP.2.0; L.2.2; V.2.6; according to de Groot, 1959). Animal behaviour was observed immediately upon recovery from anaesthesia (5–8 min after injection) and again daily up to 28 days. In addition, behaviour was also observed after apomorphine injections (1 mg kg^{-1}, i.p.). The number of complete 360° turns was recorded manually, while observing the animals in an open flat surfaced cage (40 × 25 × 10 cm) and only those animals showing tight circling responses were retained for subsequent biochemical analysis.

Animals were allowed at least one day to recover after treatments before death and biochemical or histological examination.

The rats were stunned, decapitated and the brains quickly removed and frozen in a solid CO_2–acetone mixture. The corpora striata from both hemispheres were dissected according to Glowinski & Iversen (1966). The substantia nigra were dissected after sectioning the diencephalon on an M.S.E. Pelcool Microtome, using the stereotaxic atlas of Pellegrino & Cushman (1967). Dissected brain regions from injected and non-injected brain hemispheres were retained separately for the fluorimetric determinations of GABA (Lowe, Robins & Eyerman, 1958), 5-HT and 5-hydroxyindole acetic acid (5-HIAA; Curzon & Green, 1970) and of dopamine (Shellenberger & Gordon, 1971) and homovanillic acid (HVA; Murphy, Robinson & Sharman, 1969) in the corpora striata only.

The GABA and monoamine concentrations of injected and non-injected hemispheres were compared using a Student's *t*-test.

Apomorphine HCl and pCPA were obtained from Sigma chemical company. EOS was a gift from Dr L. J. Fowler, The School of Pharmacy, University of London, Brunswick Square, London.

RESULTS

Upon recovery from anaesthesia, EOS (100 μg)-injected rats exhibited marked spontaneous contralateral circling behaviour (Fig. 1), which intensified up to 1 h, but gradually declined between 1 and 3 h after the injection. The lower concentrations of EOS (25 and 50 μg) did not produce spontaneous rotation but contralateral curvature of the head and upper body (postural asymmetry). pCPA-injected rats only showed ipsilateral postural asymmetry indistinguishable from that shown by saline-injected rats.

When apomorphine (1 mg kg^{-1}, i.p.), was injected 24 h after prior injection of EOS or pCPA, it induced marked ipsilateral circling behaviour. In the EOS-pretreated animals, the rate of apomorphine circling (expressed as a 30 min count) reached a peak at 24 h, declining consistently thereafter up to 7 days when the circling behaviour induced by daily administration of apomorphine had almost ceased (Fig. 2). In contrast, the injection of apomorphine into pCPA-treated animals elicited a more intensive circling behaviour which was maintained up to 28 days later (Fig. 2). In both types of pretreatment, the intensity of circling was apparently proportional to the pretreatment dose of EOS or pCPA.

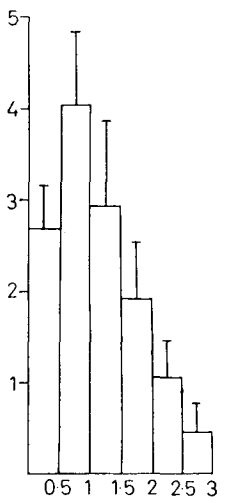

FIG. 1. Number of spontaneous contralateral circles min^{-1} (ordinate) over 30 min intervals compared with the time (h) (abscissa) after the unilateral nigral injection of EOS (100 μg).

Despite initial effects upon behaviour lasting up to 3 h, 24 h after either EOS or pCPA treatment the animals showed no postural asymmetry.

Biochemical analysis of the brains of rats having received an intranigral injection of EOS (100 μg) 24 h before death, indicated that the HVA content of the corpus striatrum of the injected hemisphere was increased (355%; $P < 0{\cdot}01$) whereas the concentration of dopamine on the same side, although

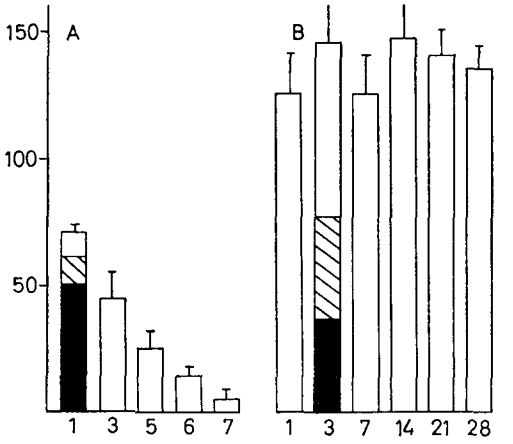

FIG. 2. Total number of ipsilateral circles (ordinate) produced by the administration of apomorphine (1 mg kg^{-1}, i.p.) during a 30 min period, following unilateral nigral injections of EOS (A) or pCPA (B), compared with the time (days) (abscissa) after the nigral injections. EOS, 100 μg open columns, 50 μg hatched column; 25 μg closed column, pCPA, 100 μg open columns; 50 μg hatched column; 25 μg closed column.

Table 1. *The effect of a single unilateral nigral injection of EOS (100 μg, 1 day) and pCPA (100 μg, 3 days) on the concentrations of 5-HT, 5-HIAA, dopamine, HVA (nmol g^{-1}) and GABA (μmol g^{-1}) in the corpus striatum.*

Treatment	5-HT		5-HIAA		Dopamine		HVA		GABA	
	Non-inj.	Inj.	Non-inj.	Inj.	Non-inj.	Inj.	Non-inj.	Inj.	Non-inj.	Inj.
EOS 1 Day								***		
	2·57 ±0·14	2·93 ±0·19	1·15 ±0·09	1·13 ±0·15	21·56 ±4·60	17·84 ±2·75	0·95 ±0·33	3·37 ±0·73	2·60 ±0·17	2·90 ±0·26
	n = 6	6	6	6	6	8	6	7	6	6
pCPA 3 Days								***		
	2·64 ±0·93	2·27 ±0·29	1·32 ±0·10	1·09 ±0·08	20·80 ±1·57	17·32 ±1·48	0·81 ±0·12	1·65 ±0·21	2·13 ±0·18	2·71 ±0·21
	n = 7	8	8	8	12	12	8	8	6	6

Mean ± s.e.m. of n determinations.
*** $P<0·01$.

reduced, was not significantly altered (17%; $P>0·5$). The contents of 5-HT, 5-HIAA and GABA were unaltered in the corpus striatum of the injected side (Table 1). Similarly only the GABA content of the injected SN was significantly increased (172%; $P<0·001$, Table 2).

Three days after pCPA (100 μg) injections, the HVA content in the corpus striatrum of the injected hemisphere was increased (240%; $P<0·01$; Table 1) this was accompanied by a slight fall in dopamine content, but no alteration of 5-HT, 5-HIAA or GABA concentrations. At this time a significant reduction of 5-HT (69%; $P<0·001$) and 5-HIAA (50%; $P<0·01$; Table 2) had occurred in the pCPA injected SN, but no significant alteration of GABA content accompanied these changes.

DISCUSSION

The elevated striatal concentration of the dopamine metabolite, HVA, following unilateral nigral injections of either pCPA or EOS suggest increased release of dopamine from the nigrostriatal bundle on the injected side. That the increase in HVA is observed only in the striatum ipsilateral to the injection argues against the possibility that the elevated HVA is either a non-specific response to surgical trauma, secondary to increased muscular activity or due to reduced clearance of HVA into cerebrospinal fluid.

After systemic administration of pCPA, Miller, Cox & others (1970) reported that 5-HT and 5-HIAA concentrations returned to control values within 10 days of pCPA treatment. In this report no apparent reversal of 5-HT depletion was observed, although pCPA was injected in a much higher local concentration than would possibly be achieved by Miller & others (1970) and a more prolonged inhibition of tryptophan hydroxylase may have occurred. The possibility exists that increased HVA concentration in the ipsilateral corpus striatum is due to more extensive damage to the SN than simply inhibition of tryptophan hydroxylase activity. Agid, Javoy & Glowinski (1973) reported that after destruction of some dopamine neurons in the SN following 6-hydroxydopamine administration, increased dopamine turnover occurred in the corpus striatum. The present observations may be due to destruction of dopaminergic neurons in the SN following injection into this region. However without measurements of dopamine concentration in the SN itself, it is difficult to substantiate this possibility and since neither GABA or 5-HT concentrations were altered after

Table 2. *The effects of a single unilateral nigral injection of pCPA (100 μg, 3 days) and EOS (100 μg, 1 day) on the concentrations of 5-HT, 5-HIAA (nmol^{-1}) and GABA (μmol g^{-1}) in the injected and control, non-injected, substantia nigra.*

5-HT		5-HIAA		GABA	
Treatment					
Non-inj.	Inj.	Non-inj.	Inj.	Non-inj.	Inj.
EOS 1 Day					***
9·44 ±1·39	9·39 ±0·74	5·21 ±0·26	6·46 ±0·68	8·32 ±1·09	14·33 ±1·68
n = 4	4	3	4	8	8
pCPA 3 Days	****		**		
10·17 ±1·65	3·16 ±0 45	5·36 ±1 72	2·71 ±0·99	8·18 ±0·39	10·8 ±1·36
n = 8	8	6	6	6	6

Mean ± s.e.m. of n determinations.
** $P<0·05$. *** $P<0·01$. **** $P<0·001$.

pCPA or EOS injections respectively, it is unlikely that gross morphological damage occurred after such injections.

It has now been widely reported that unilateral injections of GABA receptor agonists into one SN produce contralateral rotation (Waddington, 1977; Oberlander, Dumont & Boissier, 1977; Scheel-Krüger, Arnt & Magelund, 1977). In this report, EOS, a specific inhibitor of GABA transaminase (Fowler & John, 1972) produced spontaneous contralateral rotation, presumably by GABA receptor activation in the SN. However, more importantly, at a time when nigral GABA concentrations were significantly increased, a directly acting dopamine agonist, apomorphine, produced ipsilateral rotation (Fig. 1 and Fig. 2; Dray & others, 1975).

If GABA is an inhibitory transmitter in the SN which modulates the nigrostriatal pathway directly, then application of GABA-agonists or elevation of GABA content, should increase inhibition on SN neurons and reduce nigrostriatal impulse flow. This would result in spontaneous ipsilateral rotation and contralateral rotation after an apomorphine challenge, because of the production of ipsilateral striatal dopamine receptor supersensitivity. However, the opposite effect is observed. Biochemical evidence, presented here, indicates that an increase in nigrostriatal impulse traffic occurs. Similarly, if 5-HT were an excitatory influence modulating the nigrostriatal pathway, then functionally reducing 5-HT activity within the SN, as after pCPA injections (Tables 1, 2), should reduce the nigrostriatal impulse. This should result in reduced HVA and increased dopamine concentrations ipsilaterally, accompanied by contralateral rotation after apomorphine administration. However, the evidence presented here indicates that ipsilateral rotation results after apomorphine challenge and pCPA pretreatment. Unlike GABA, 5-HT appears to have a direct inhibitory influence on the nigrostriatal impulse flow.

The possibility now exists, therefore, that descending GABAergic striatonigral fibres indirectly modulating the nigrostriatal pathway may form synaptic connections with non-GABA releasing inhibitory interneurons within the zona reticulata. Since reducing the functional activity of 5-HT within the SN releases inhibitory restraints on the nigrostriatal pathway and that 5-HT containing terminals make axodendritic connections with dopamine neurons (Parizek, Hassler & Bak, 1971), the possibility exists that GABA and 5-HT may be intimately related within the zona reticulata in controlling the nigrostriatal impulse flow.

REFERENCES

Agid, Y., Javoy, F. & Glowinski, J. (1973). *Nature New Biol.*, **245**, 150–151.
Costall, B. & Naylor, R. J. (1974). *Eur. J. Pharmac.*, **29**, 206–222.
Curzon, G. & Green, A. R. (1970). *Br. J. Pharmac.*, **39**, 653–655.
De Groot, J. (1959). *Verh. K. Ned. Akad. Wet.*, **52**, 14–39.
Dray, A., Fowler, L. J. Oakley, N. R., Simmonds, M. A. & Tanner, T. (1975). *Br. J. Pharmac.*, **55**, 288P.
Dray, A., Gonye, T. J., Oakley, N. R. & Tanner, T. (1976). *Brain Res.*, **113**, 45–57.
Dray, A. & Straughan, D. W. (1976), *J. Pharm. Pharmac.*, **28**, 400–405.
Feltz, P. (1971). *Can. J. Physiol. Pharmac.*, **49**, 1113–1115.
Fowler, L. J. & John, R. A. (1972). *Biochem. J.*, **130**, 569–573.
Glowinski, J. & Iversen, L. L. (1966). *J. Neurochem.*, **13**, 655–669.
Hadju, F., Hassler, R. & Bak, I. J. (1973). *Z. Zellforsch mikrosk. Anat.*, **146**, 207–221.
Kim, J. S., Bak, I. J., Hassler, R. & Okada, Y. (1971). *Exp. Brain Res.*, **14**, 95–104.
Lowe, I. P., Robins, E. & Eyerman, G. S. (1958). *J. Neurochem.*, **3**, 8–18.
Miller, F. P., Cox, R. H., Snodgrass, W. R. & Maickel, R. P., (1970), *Biochem. Pharmac.*, **19**, 435–442.
Murphy, G. F., Robinson, D. & Sharman, D. F. (1969). *Br. J. Pharmac.*, **36**, 107–115.
Oberlander, C., Dumont, C. & Boissier, J. R. (1977). *Eur. J. Pharmac.*, **43**, 389–390.
Parizek, J., Hassler, R. & Bak, I. J. (1971). *Z. Zellforsch mikrosk. Anat.*, **115**, 137–148.
Pellegrino, L. J. & Cushman, A. J. (1967). *A Stereotaxic atlas of the rat brain.*
Precht, W. & Yoshida, M. (1971). *Brain Res.*, **32**, 229–233.
Scheel-Krüger, J., Arnt, J. & Magelund, G. (1977). *Neurosci. Letts.*, **4**, 351–356.
Shellenberger, M. K. & Gordon, J. H. (1971). *Analyt. Biochem.*, **39**, 356–372.
Ungerstedt, U. (1971). *Acta. physiol. scand.*, **82**, *Suppl.*, **367**, 69.
Waddington, J. L. (1977). *Br. J. Pharmac.*, **60**, 263P.