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Circling Behavior in Rodents Following an Imbalance of Basal Ganglia Gaba Concentrations[1,2]

R. W. HORTON AND C. J. PYCOCK[3]

University Department of Neurology, Institute of Psychiatry, Denmark Hill London, SE5 8AF, England

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HORTON, R. W. AND C. J. PYCOCK. *Circling behaviour in rodents following an imbalance of basal ganglia GABA concentrations.* PHARMAC. BIOCHEM. BEHAV. 6(5) 493–497, 1977. – Unilateral focal injection of the GABA – transaminase inhibitor, ethanolamine O-sulphate, into one substantia nigra or globus pallidus of rats or the striatum of mice induces spontaneous and drug-induced circling behaviour. Circling parallels the imbalance of GABA concentrations between the injected and the noninjected side of the brain, being most striking on Day 1 and 3, and non-existent by Day 7. Increases in GABA concentration were demonstrated in areas distant from the injection site, on both the injected and noninjected side of the brain presumably due to diffusion of the ethanolamine O-sulphate. This diffusional effect made it impossible to define the exact site of GABA and dopamine interaction.

γ-aminobutyric acid Ethanolamine O-sulphate Basal ganglia Spontaneous and drug-induced circling behaviour γ-aminobutyric acid/dopamine interaction

BIOCHEMICAL, histochemical and electrophysiological studies have implicated a neurotransmitter role of γ-aminobutyric acid (GABA) in the brain (for reviews, [15,20]). GABA is found in high concentrations in the dopamine-rich areas of the basal ganglia although the exact functional relationships between these two neurotransmitters are still not clear. Recent reports have suggested that basal ganglia GABA dysfunction may be implicated in certain neurological disorders such as Huntington's chorea [2,18].

An imbalance of basal ganglia dopaminergic activity in rodents results in rotational behaviour, the animal turning in circles away from the side of higher activity [1,22]. The classical rotating rat is produced by lesioning one nigro-neostriatal dopaminergic pathway, leaving a striatum void of dopamine-containing terminals, but with intact dopamine receptors. Such animals will rotate towards the lesioned side when given an indirectly-acting dopamine agonist, such as amphetamine, which causes release of dopamine from the intact striatum, and away from the lesioned side when given directly-acting agonists, such as apomorphine, due, it is suggested, to preferential stimulation of the supersensitive denervated receptors. [22].

Recently, Dray, Oakley and Simmonds [7] reported drug-induced ipsilateral rotation in rats following focal elevation of GABA concentrations in one substantia nigra. Tarsy and colleagues [21] have reported contralateral turning in rats following unilateral intra-nigral injection of the GABA receptor blocking agent, picrotoxin. We have further explored the possibilities of inducing circling in rodents by unilateral injection of ethanolamine O-sulphate, an irreversible, active site directed inhibitor of GABA-transaminase [9], at various sites within the basal ganglia. We have measured regional brain GABA concentrations in an attempt to relate the observed rotation to biochemical changes.

METHOD

Injection Procedure

Male Sprague-Dawley rats (180–200 g) were anaesthetised with chloral hydrate (300 mg/kg, IP) and immobilised in a Kopf stereotaxic frame. Unilateral injections of ethanolamine O-sulphate (100 μg in 1 μl saline over a 2 min period) were made with a Hamilton syringe into either the substantia nigra (A + 2.4, L 1.8, V – 2.6) or globus pallidus (A + 6.5, L 2.5, V – 1.0) (co-ordinates from König and Klippel, [12]). Control animals received 1 μl saline into the same sites.

Male Swiss 'S' mice received unilateral injections of ethanolamine O-sulphate (100 μg in 2.5 μl saline or saline

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[3] Present address: Department of Pharmacology, Medical School, University of Bristol, Bristol BS 8 ITD, England.

alone) into the striatum by the free-hand injection technique as previously described [19].

Behavioural Testing

Following recovery from anaesthesia, animals were observed for postural asymmetries, spontaneous circling and drug-induced circling activity at 1, 3 and 7 days after operation. For behavioural testing, rats were placed in plastic cages measuring 35 × 25 cm, and the mice in boxes measuring 12 × 12 cm. Circling rates were assessed as the number of complete turns made by an animal in a one-minute period. Spontaneous activity was recorded after a 5 min habituation period. Drug-induced circling behaviour was measured 30 min following amphetamine (4 mg/kg, IP) or 15 min after apomorphine (0.5 mg/kg, SC).

Biochemical Analysis

Other animals injected with ethanolamine O-sulphate or saline into the same site as for behavioural testing were killed on Days 1, 3, and 7 after operation for the measurement of regional GABA concentrations. Animals were killed by decapitation, and the various brain regions from injected and noninjected sides were rapidly dissected out and frozen in liquid nitrogen. Brain areas from the ethanolamine O-sulphate treated groups were always dissected in the same order; the time interval from decapitations to freezing the last sample did not exceed 60 sec. For substantia nigra rats, mesolimbic (nucleus accumbens + tuberculum olfactorium), striatal and nigral samples were taken, and for globus pallidus rats, mesolimbic, frontal cortex and pallidal/posterior caudate nucleus regions were taken. Only the striatum was dissected out from mice.

GABA Estimation

Frozen tissue samples were quickly weighed and homogenised in ice-cold 0.6 N perchloric acid containing 1 mm EDTA. Perchloric acid extracts were neutralised with 2 N potassium hydroxide, potassium perchlorate removed by centrifugation, and samples diluted to a known volume. Aliquots of samples plus appropriate tissue blanks were assayed for GABA concentrations by the enzymatic method of Graham and Aprison [10].

Histology

Animals selected at random were killed and the brains fixed for a week in formal saline. Identification of the injection site was verified by both macroscopic and histological techniques.

Drugs

Ethanolamine O-sulphate was synthesized by the method of Lloyd, Tudball and Dodgson [13]. Dexamphetamine sulphate (Smith, Kline and French) and apomorphine hydrochloride (Evans Medical) were dissolved in saline. Weights of drugs are expressed in terms of the salt.

Statistical Analysis

Changes in GABA concentrations and circling rates were statistically compared using Student's *t*-test.

RESULTS

Circling Behaviour

None of the control animals injected with saline into any of the sites studied within the basal ganglia exhibited any degree of postural asymmetry, spontaneous circling activity or drug-induced circling behaviour on the first or subsequent days after operation.

The results of both spontaneous, amphetamine and apomorphine-induced circling behaviour in all three ethanolamine O-sulphate injected animal groups are presented in Fig. 1. Unilateral focal injections of ethanolamine O-sulphate into either the substantial nigra or globus pallidus of rats or into the striatum of mice, resulted in animals that showed both spontaneous and drug-induced circling behaviour towards the injected side on both Days 1 and 3 after operation. Amphetamine (4 mg/kg, IP) and apomorphine (0.5 mg/kg, SC) both induced ipsiversive circling of similar intensity on Day 1 and Day 3 (a mean rate of 5–6 turns per min in all three animals group). By Day 7 both spontaneous and drug-induced circling activities were very much reduced (under one turn per min) or non-existent. On Days 1 and 3, drug-induced circling behaviour was always accompanied by a tight ipsiversive body asymmetry in all three ethanolamine O-sulphate injected animals groups.

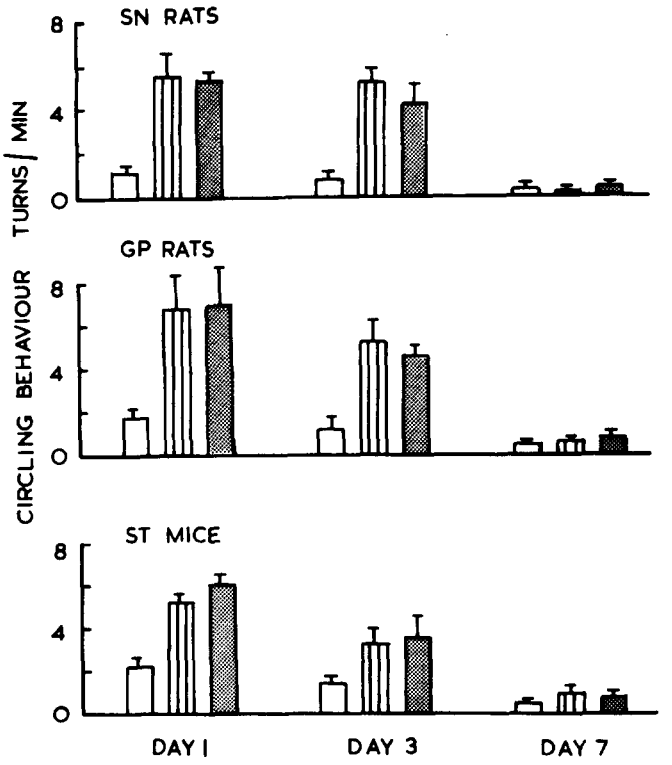

FIG. 1. Spontaneous and drug-induced circling rates following focal injection of ethanolamine O-sulphate into one substantia nigra (SN) or globus pallidus (GP) of rats or the striatum (ST) of mice on Days 1, 3 and 7 after operation. All circling was observed towards the injected side and was recorded as the number of turns completed in a 1 min period. Circling was recorded spontaneously (open columns) 15 min after apomorphine (0.5 mg/kg, SC), (striped columns) or 30 min after amphetamine (4 mg/kg, IP) (dotted columns). Vertical bars denote standard errors. Number of observations for each point was 6.

TABLE 1

THE TIME COURSE OF CHANGES IN REGIONAL BRAIN GABA CONCENTRATIONS FOLLOWING UNILATERAL FOCAL INJECTIONS OF ETHANOLAMINE O-SULPHATE INTO THE BASAL GANGLIA OF RATS AND MICE

		ML	St	SN	Cortex	GP	ML	St	SN	Cortex	GP
Control Rats							2.67 ± 0.34	1.56 ± 0.12	2.94 ± 0.29	1.15 ± 0.10	2.21 ± 0.20
		Injected Side					Noninjected Side				
		4.91 ± 1.09	2.31 ± 0.13	8.64 ± 1.02			3.64 ± 0.55	1.92 ± 0.24	3.18 ± 0.42		
	DAY 1	135%	120%	272%†							
		4.31 ± 0.28	4.93 ± 0.31	8.81 ± 0.96			4.33 ± 0.73	2.57 ± 0.14	5.15 ± 0.77		
SN Rats	DAY 3	99.5%	192%†	171%*							
		3.26 ± 0.16	2.20 ± 0.13	2.67 ± 0.21			3.37 ± 0.45	1.93 ± 0.12	3.36 ± 0.56		
	DAY 7	97%	114%	80%							
		10.26 ± 1.19			5.40 ± 0.18	6.52 ± 0.89	3.89 ± 0.99			2.98 ± 0.26	3.81 ± 0.37
	DAY 1	264%*			181%†	171%*					
		11.83 ± 0.92			6.72 ± 0.53	16.97 ± 6.8	8.85 ± 0.71			3.59 ± 0.21	5.57 ± 0.59
GP Rats	DAY 3	134%*			187%†	305%†					
		4.70 ± 0.27			1.95 ± 0.08	3.27 ± 0.20	4.46 ± 0.48			1.58 ± 0.04	2.68 ± 0.15
	DAY 7	105%			123%‡	122%‡					
			7.89 ± 1.32					4.41 ± 0.87			
	DAY 1		179%*								
			5.25 ± 0.66					3.21 ± 0.64			
St Mice	DAY 3		164%								
			2.89 ± 0.38					2.85 ± 0.42			
	DAY 7		101%								

Rats and mice were killed at the stated times following focal unilateral injection of ethanolamine O-sulphate or saline on Day 0. Brain areas are denoted as: -mesolimbic (ML), Striatum (St), Substantia nigra (SN), frontal cortex (cortex) and pallidum/posterior caudate (GP) Brain GABA concentrations (μ moles/g) are expressed as means ± SEM (n = 6–10) with the % increase on the injected side compared to the non-injected side. The significance of the differences between the injected and non-injected side (Student's t test) is denoted by * $p<0.05$, ‡ $p<0.0005$. Rats injected with saline into one substantia nigra or one globus pallidus showed no significant differences in GABA concentrations between the injected and non-injected sides or between the sites of injection and are thus presented as a combined control group.

Observation of the ethanolamine O-sulphate injected rat groups awakening from anaesthesia showed that these animals consistently turned tightly away from the injected side. Such contraversive circling lasted only a few hours.

GABA Concentrations

GABA concentrations in various brain regions from injected and noninjected sides after focal unilateral injections of ethanolamine O-sulphate in the three animal groups are shown in Table 1, together with regional GABA concentrations in control (saline-injected) rats.

Unilateral injections of ethanolamine-O-sulphate into the substantia nigra resulted in a 3-fold increase in GABA concentration in this region on the injected side compared with the noninjected side on Day 1 after operation ($p<0.005$). This elevation fell to 2-fold by Day 3 ($p<0.05$) and there was no significant difference between the injected and noninjected sides by Day 7. Compared to saline injected controls the GABA concentration in the substantia nigra on the noninjected side was increased (maximally, 175% on Day 3, $p<0.01$) but was not significantly different on Days 1 and 7. The striatal GABA concentration on the ethanolamine-O-sulphate injected side was increased at all times compared to saline injected controls, but an imbalance between the ethanolamine O-sulphate injected and noninjected sides was only seen on Day 3 ($p<0.005$). There was no significant imbalance of GABA concentrations between the injected and noninjected sides in the mesolimbic areas at any time studied following focal injection of ethanolamine O-sulphate into one substantia nigra. The mesolimbic GABA concentration of the non-injected side was, however, significantly increased compared to control saline-injected values on Day 3 (162%, $p<0.05$).

Unilateral injection of ethanolamine-O-sulphate into the globus pallidus resulted in a 2-fold increase in GABA concentration in this region on the injected side compared to the noninjected side on Day 1 after operation ($p<0.05$). and a 3-fold increase on Day 3 ($p<0.005$). A small, but statistically significant imbalance of pallidal GABA concentration remained at Day 7 ($p<0.05$). The concentration of GABA in the globus pallidus of the noninjected side was significantly increased compared to saline injected controls on Days 1 and 3.

Increase in cortical and mesolimbic GABA concentrations were seen in both injected and noninjected sides compared to saline-treated controls. However, there was a significant imbalance between the ethanolamine O-sulphate injected and noninjected sides (maximally 264% increase on the injected side in the mesolimbic area on Day 1 and maximally 87% increase on the injected side in the cortical area on Day 3.

Unilateral injection of ethanolamine O-sulphate into one striatum of mice caused an 80% increase in GABA levels ($p<0.05$) on the injected side as compared with the noninjected side on Day 1. On Day 3 this was reduced to a 64% increase ($p<0.05$) and by Day 7 there was no difference between striatal GABA concentrations of the two sides.

Histological Observations

Histological examination of tissue sections verified that all injections were within the target area. Apart from the expected slight gliosis defining the needle tract, no tissue damage was observed.

DISCUSSION

Unilateral injection of the specific GABA-T inhibitor ethanolamine O-sulphate into the substantia nigra, globus pallidus, or striatum of rodents results in an animal that circles towards the injected side both spontaneously and after either amphetamine or apomorphine. This behavioural

effect was absent in control animals previously injected with saline into the various regions of the basal ganglia. This, together with the histological evidence of minimal tissue damage, suggests that the circling activity is a true drug-induced effect. Similarly, the fact that circling activity was very much diminished or absent 7 days after the intracerebral injection of ethanolamine O-sulphate dismisses the possibility of permanent tissue damage.

Circling activity paralleled an imbalance in brain GABA concentrations between the injected and noninjected sides, suggesting a GABA-mediated effect. The correlation was strongest in the rats injected into the substantia nigra, there being a marked imbalance of GABA concentrations in this region on the injected side compared to the noninjected side at Days 1 and 3, and no difference by Day 7. The only other imbalance observed in the regions measured was a significant GABA increase in the striatum on the injected side at Day 3, which itself probably contributes to the turning phenomenon, as suggested from the experiments in the mice. However, there was no significant imbalance in striatal GABA at Day 1 in the substantia nigra injected rats when turning was of equal intensity to that on Day 3. The biochemical and behavioural correlation in the globus pallidus injected rats is less clear. In these animals there was a significant imbalance between injected and noninjected sides in the three regions measured at each time (except the mesolimbic areas at Day 7) although by Day 7 the increases were only of the order of 20%. Therefore spread of ethanolamine O-sulphate induced GABA elevation was more prominent in the pallidal rats than in the nigral animals. Such a difference is probably due to two factors; the relative distances involved between the structures, for example, the globus pallidus and mesolimbic areas (approximately 3 mm) or the substantia nigra and mesolimbic areas (approximately 7 mm), and secondly, the closer proximity of the ventricular system to the globus pallidus as compared with the substantia nigra may, at least in part, facilitate such a diffusion. Injection of ethanolamine O-sulphate into one side of the brain not only increased GABA concentrations on that side as compared to the noninjected side, but also elevated GABA concentrations on both sides of the brain when compared to saline-injected control animals. Unfortunately, this apparent diffusion of the ethanolamine O-sulphate effect may limit its use as an elegant research tool, with which to study the interaction of GABA with other neurotransmitter systems. However, our results support the suggestion that circling behaviour is observed following an imbalance of GABA concentrations between two sides of the brain, rather than being directly related to absolute increased in GABA concentrations.

In the rotating model of Ungerstedt [22] where degeneration of one nigro-neostriatal dopaminergic pathway is achieved by the direct injection of 6-hydroxydopamine into the cell bodies of the substantia nigra, amphetamine causes rotation towards the lesioned side while apomorphine causes rotation towards the intact side. However, following unilateral elevation of GABA both amphetamine and apomorphine induce rotation in the same direction, i.e. towards the injected side. Such a result suggests that these agents are both acting on the noninjected side. Therefore it is likely that the elevation of GABA has rendered one side of the brain unresponsive to dopaminergic stimulation. Similarly, electrolytic lesions of one nigro-neostriatal pathway or of one globus pallidus produces an animal that circles towards the lesioned side in response to both directly and indirectly acting dopamine agonists [5,16]. At the former site it is likely that the electrolesion has not only destroyed the ascending nigro-neostriatal dopaminergic pathway but also a second neuronal system, perhaps a nondopaminergic nigro-neostriatal tract or a strio-pallidal efferent pathway, required for expression of circling behaviour resulting from stimulation of dopaminergic receptors in the denervated striatum [4]. The similarity of the electrolesion and ethanolamine O-sulphate effect in rat substantia nigra has been reported [6].

A number of studies have provided evidence for the existence of inhibitory GABAminergic strio-nigral and pallido-nigral pathways in rat brain [11, 14, 17]. Such pathways may serve to regulate the activity of the ascending dopaminergic nigro-neostriatal tract. Thus an elevation of GABA levels in one substantia nigra may produce decreased dopaminergic nigro-neostriatal transmission on that side, as remarked by Dray *et al.* [7] which might well account for amphetamine-induced ipsilateral rotation, but fails to explain apomorphine-induced ipsilateral rotation. Apomorphine is believed to act directly at dopamine receptors [8] and thus should be predominantly independent of presynaptic nerve activity.

The present demonstration of a spread of elevated GABA levels from the region of the substantia nigra to other areas, may account for the apomorphine effect. Thus, elevated GABA may cause local inhibition of dopamine receptor stimulation in the striatum itself or possibly at other levels of the strio-efferent pathway such as the globus pallidus through which dopamine-dependent circling behaviour is expressed. Alternatively, as suggested by Dray and colleagues [6], the behavioural observations may be the results of an increase in nigro-neostriatal dopaminergic transmission caused by GABA-mediated inhibition of non-GABA inhibitory neurones within the region of the substantia nigra on the injected side.

In agreement with Dray and his colleagues [7] we also noted a transient contralateral asymmetry, together with some circling, when the rats awoke from anaesthesia. We too have no definite explanation for this effect other than a nonspecific stimulation of certain neurones.

It can be concluded that an imbalance of GABA concentrations in the rodent brain induces circling behaviour to dopamine agonists. Such a result suggests that eleveated GABA concentrations inhibit dopaminergic mechanisms, but it is difficult to assess at which level such control is apparent. From these experiments it is not possible to say whether modification of dopamine function occurs at the level of the dopamine receptor, at the level of a striatal or nigral interneurone, or perhaps, at the level of the globus pallidus. The recent work of Cools and Jannsen [3] supports this conclusion as they found that GABA can completely suppress the effects induced by either dopamine or apomorphine in certain regions of cat caudate nucleus.

The results of our experiments underline the problems of focally injecting ethanolamine O-sulphate into the brain. We can only assume that the diffuse increase in GABA concentrations is entirely related to the spread of ethanolamine O-sulphate away from the injection site rather than secondary changes of transmitter turnover. Previously such a spread of the ethanolamine O-sulphate induced effect has not been reported: for example, it is surprising that no circling behaviour was observed after dopamine agonists in rats in which ethanolamine O-sulphate was injected immediately above the substantia nigra [6].

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