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REGIONAL CHANGES IN THE CONCENTRATIONS OF CEREBRAL MONOAMINES AND THEIR METABOLITES AFTER ETHANOLAMINE-*O*-SULPHATE INDUCED ELEVATION OF BRAIN γ-AMINOBUTYRIC ACID CONCENTRATIONS

CHRISTOPHER PYCOCK* and ROGER HORTON†
*Department of Pharmacology, Medical School, University of Bristol, Bristol, BS8 1TD and †Department of Neurology, Institute of Psychiatry, Denmark Hill, London, SE5 8AF, England

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Abstract—The effect of ethanolamine-*O*-sulphate induced elevation of cerebral GABA concentrations on monoamine and their metabolites levels has been studied in various regions of the rat brain. Increased GABA concentrations were associated with a decrease in turnover of dopamine in limbic regions: striatal dopamine was not significantly affected. An increased turnover of 5-hydroxytryptamine was also observed in other brain areas. Increased cerebral GABA concentrations had no effect on regional noradrenaline turnover. The possible sites of interaction between the neurotransmitters are discussed.

Although γ-aminobutyric acid (GABA) is now widely accepted as an inhibitory neurotransmitter in the central nervous system, few GABAergic pathways are clearly defined. Electrophysiological, biochemical and pharmacological evidence suggests that the ascending dopaminergic (DA) nigro-striatal pathway may be regulated by a descending pathway, originating in the strio-pallidal complex, which utilises GABA as a neurotransmitter [1, 2]. Pharmacological evidence also suggests that DA pathways can influence the activity of GABA neurones. The DA receptor agonist apomorphine can increase GABA turnover in the striatum, the limbic areas and DA-cell body rich areas of the ventral mid-brain [3] while DA blocking neuroleptic drugs are reported as decreasing GABA turnover in certain brain nuclei [4]. Thus evidence is accumulating to support the concept of a functional interaction between DA and GABA within the DA rich areas of the brain.

Also GABA is often found in high concentrations in areas of the brain rich in the other monoamine neurotransmitters 5-hydroxytryptamine (5-HT) and noradrenaline (NA); for example in limbic areas and the hypothalamus [5]. A possible interaction between GABA and these neurotransmitters in those DA-deficient brain areas seems worthy of investigation.

Ethanolamine-*O*-sulphate (EOS) is a specific active-site-directed irreversible inhibitor of GABA-transaminase [6], the enzyme which catalyses the further metabolism of GABA [7]. We have injected EOS intracerebroventricularly and studied the effects of the resulting elevation of cerebral GABA concentrations on the concentration of DA, 5-HT and NA and their metabolites in specific regions of the rat brain.

MATERIALS AND METHODS

Male and female Sprague–Dawley rats (200–250 g) were anaesthetised with chloral hydrate (300 mg/kg, i.p.) and immobilised in a Kopf stereotaxic frame. EOS (100 μg in 1.5 μl saline) or an equivalent volume of saline was injected into the lateral ventricle (co-ordinates from König and Klippel [8]). Control and drug treated rats were divided into three groups. One group was used for the estimation of monoamine concentrations (DA, 5-HT, NA and 5-hydroxyindoleacetic acid (5-HIAA)); a second group for the estimation of monoamine metabolite concentrations, (homovanillic acid, (HVA), 3,4-dihydroxyphenylacetic acid, (DOPAC) and 4-hydroxy, 3-methoxyphenylethylene glycol sulphate (MOPEG-SO_4)); and a third group for the estimation of GABA concentrations. Animals were killed by cervical dislocation 24 hr after the injection of EOS or saline: a time at which the behavioural akinetic state and the increase in cerebral GABA concentrations were maximal.

The brain was removed intact from the skull. The hypothalamus was scooped out with curved forceps (mean weights ± SEM, 23.5 ± 0.6 mg, n = 24). Left and right olfactory tubercules were dissected out with the underlying accumbens nuclei ('limbic forebrain' 80.2 ± 3.9 mg, n = 36). The cerebellum (278.8 ± 5.7 mg, n = 36) was removed by blunt dissection at the level of the posterior colliculus. A transverse section caudal to the posterior colliculus and at the caudal tip of the IVth ventricle yielded the pons (163.0 ± 5.1 mg, n = 36). An approximately 1 mm thick slice was taken from the region of the fronto-parietal cortex (277.5 ± 10.0 mg, n = 36). The remaining cerebral mantles were peeled back laterally and the two striata pooled (69.2 ± 3.2 mg, n = 36). The central portion of the mesencephalon was removed by cutting transversely at the caudal point of the hypothalamus [9] and the ventral portion containing the substantia nigra was taken for assay ('ventral midbrain' 55.2 ± 2.0 mg, n = 36).

Chemical analyses were performed on areas from single brains by previously published proce-

dures—5-HT and 5-HIAA [10], NA [11], DA [12] and MOPEG-SO_4 [13]. HVA and DOPAC were isolated on Sephadex columns and assayed fluorimetrically [14]. For the estimation of GABA concentrations, brain areas were rapidly dissected, in the same order for control and drug-treated animals, and placed in liquid nitrogen. The time interval from killing the animal to freezing the last sample of tissue did not exceed 90 sec. GABA was estimated in deproteinised neutralised extracts [15].

Monoamine and monoamine metabolite concentrations were estimated by comparison with a range of standards carried through the extraction and assay procedure. In cases where column purification was used the values have not been corrected for recovery, which was 50–60 per cent for DA and better than 80 per cent for HVA, DOPAC and MOPEG-SO_4.

RESULTS

EOS treated rats were akinetic and sat flexed, although their hind limbs extended when picked up. They exhibited limb rigidity and occasional resting tremor. No abnormal behavioural effects were observed in rats which received an equivalent volume of saline intraventricularly.

The regional concentration of neurotransmitter and neurotransmitter metabolites determined in control rats is shown in Table 1. Highest concentrations of DA were found in the striatum, the limbic region and the hypothalamus. NA concentrations were highest in the hypothalamus, the limbic region and pons. 5-HT concentrations were highest in the hypothalamus, the limbic region and the striatum. GABA concentrations were highest in the hypothalamus and the limbic region and lowest in spinal cord.

After EOS treatment GABA concentrations were increased in all brain regions studied. The increases were similar in all regions except the spinal cord (Table 2). DA concentrations were apparently increased (8–34 per cent) in all areas except the pons and spinal cord. The increase was greatest in the striatum, but none of the increases reached statistical significance. HVA and DOPAC concentrations were decreased in all areas studied, but only the decrease in limbic DOPAC concentration was statistically significant ($P < 0.05$) (Table 2). No significant changes were seen in NA or MOPEG-SO_4 concentrations. 5-HT concentrations were not significantly changed but 5-HIAA concentrations were increased 72 per cent in ventral mid-brain, 78 per cent in pons and 116 per cent in the cortex (Table 2).

DISCUSSION

The changes in steady-state concentration of transmitter metabolites is often taken as an index of transmitter turnover within the central nervous system. Using this premise, the present results demonstrate that EOS-induced elevation of GABA concentrations in the rat brain leads to a decrease in limbic DA turnover (as reflected by a fall in DOPAC concentrations) and to regional changes in 5-HT turnover (5-HIAA levels). No differences in NA or its metabolite MOPEG-SO_4 concentrations were noted.

A decrease in DA turnover in the limbic region following elevation of GABA concentrations might suggest a stronger GABA-mediated control of limbic DA neurones than of striatal DA neurones. This conclusion agrees with a previous study in which different drugs from EOS were used to influence GABA mediated transmission and different techniques were used to estimate DA turnover [16]. However, other studies have reported similar effects on both striatal and limbic DA turnover induced by drugs which influence GABA-mediated neurotransmission [17–19]. The study most easily compared with the present one is that reported by Huot and colleagues [19] in which elevation of GABA concentrations, induced by another specific active-site-directed inhibitor of GABA-transaminase, γ-acetylenic GABA (GAG, [20]) decreased DA turnover in both striatum and limbic areas. Such differences may be related to the faster rise in GABA concentrations (6 hr after GAG, 24 hr after EOS) and to the extent of the GABA increase (6-fold after GAG, 4-fold after EOS). In the present study there was a tendency towards a decreased turnover of DA in the striatum, (as judged by the decrease of striatal HVA and DOPAC concentrations) although the decrease did not reach significance.

The site or sites at which increased GABA concentrations (supposedly through enhanced GABA mediated transmission) act to decrease DA turnover are not known. One possible site is at the DA cell body rich area of the ventral mid-brain, since descending pathways from forebrain, which possibly utilise GABA as the neurotransmitter substance [1, 2] can influence the activity of the ascending DA pathways. However, electrophysiological work [21] and behavioural studies [22] suggest that facilitating GABAergic transmission in the zona reticulata of the substantia nigra in fact increases the activity of the ascending nigro-striatal neurones. Thus, such an action of elevated nigral GABA concentrations would not be expected to lead to the decreased DA turnover as observed. However, there is probably more than one site within the substantia nigra where the GABA:DA interaction may occur. For example when injected unilaterally into the substantia nigra of rats EOS leads to ipsiversive turning [23, 24] whilst the putative GABA agonist muscimol is reported to cause contraversive circling [22, 25]. Such opposing results would suggest differential GABA:DA interactions within the substantia nigra.

An alternative possibility for the site of GABA:DA interaction may be at the level of the DA terminals within the striatum and limbic areas. Blockade of GABAergic neurones within the striatum by bicuculline or picrotoxin has been shown to enhance the release of DA while infusion of GABA diminished spontaneous DA release [26]. This biochemical relationship receives behavioural support whereby focal injection of EOS into the nucleus accumbens, with subsequent elevation of GABA concentration, inhibits the hyperactivity

Table 1. Regional neurotransmitter and neurotransmitter metabolite concentrations in control rat brain

	Striatum	Limbic	Cortex	Hypothalamus	Cerebellum	Pons	Ventral Mid-Brain	Spinal Cord
DA	2.88 ± 0.23	2.77 ± 0.35	0.25 ± 0.06	2.26 ± 0.45	0.14 ± 0.05	0.41 ± 0.19	0.36 ± 0.12	0.46 ± 0.16
HVA	0.42 ± 0.04	0.31 ± 0.01					0.34 ± 0.04	
DOPAC	1.17 ± 0.14	1.25 ± 0.10					0.61 ± 0.15	
NA	0.22 ± 0.03	0.84 ± 0.07	0.32 ± 0.02	2.32 ± 0.24	0.28 ± 0.05	0.54 ± 0.07	0.42 ± 0.05	0.27 ± 0.04
MOPEG-SO_4			0.22 ± 0.02		0.21 ± 0.03	0.44 ± 0.05		
5HT	0.73 ± 0.08	0.76 ± 0.08	0.35 ± 0.03	0.87 ± 0.09	0.05 ± 0.02	0.46 ± 0.06	0.46 ± 0.07	0.45 ± 0.06
5HIAA	0.55 ± 0.05	0.47 ± 0.08	0.17 ± 0.03	0.65 ± 0.07	0.08 ± 0.01	0.32 ± 0.03	0.40 ± 0.03	0.28 ± 0.04
GABA	1.43 ± 0.13	2.37 ± 0.08	0.93 ± 0.09	2.98 ± 0.36	0.75 ± 0.06	0.82 ± 0.13	1.70 ± 0.19	0.42 ± 0.04

Rats were killed, the brains dissected and tissue samples prepared and analysed as in the methods section. Results are expressed as mean ± S.E.M. for 6 determinations. GABA concentration is expressed as μ moles/g, the remainder as μg/g wet weight tissue.

Table 2. Regional neurotransmitter and neurotransmitter metabolite concentrations in rat brain after EOS administration

	Striatum	Limbic	Cortex	Hypothalamus	Cerebellum	Pons	Ventral Mid-Brain	Spinal Cord
DA	3.86 ± 0.97	3.01 ± 0.56	0.27 ± 0.08	2.47 ± 0.52	0.17 ± 0.05	0.40 ± 0.09	0.39 ± 0.09	0.31 ± 0.05
	134%	109%	108%	109%	121%	98%	108%	67%
HVA	0.36 ± 0.04	0.28 ± 0.02					0.29 ± 0.02	
	84%	91%					85%	
DOPAC	0.94 ± 0.17	0.82 ± 0.15					0.63 ± 0.07	
	80%	66%*					103%	
NA	0.19 ± 0.03	0.93 ± 0.11	0.33 ± 0.04	1.85 ± 0.24	0.21 ± 0.02	0.57 ± 0.06	0.31 ± 0.04	0.27 ± 0.04
	87%	110%	103%	80%	75%	105%	73%	98%
MOPEG-SO_4			0.30 ± 0.06		0.23 ± 0.05	0.48 ± 0.09		
			136%		110%	107%		
5HT	0.72 ± 0.09	0.99 ± 0.13	0.30 ± 0.03	0.88 ± 0.12	0.07 ± 0.02	0.59 ± 0.19	0.50 ± 0.10	0.42 ± 0.08
	99%	130%	86%	100%	137%	128%	110%	94%
5HIAA	0.67 ± 0.06	0.61 ± 0.05	0.36 ± 0.10	0.73 ± 0.17	0.13 ± 0.03	0.57 ± 0.12	0.68 ± 0.08	0.35 ± 0.05
	120%	128%	216%*	112%	156%	178%*	172%*	127%
GABA	6.15 ± 0.40	9.44 ± 1.99	3.56 ± 0.36	12.39 ± 0.91	3.32 ± 0.54	4.03 ± 0.66	5.00 ± 0.61	0.70 ± 0.23
	430%†	398%†	383%†	416%†	443%†	383%†	294%†	167%

Rats were injected with EOS (100 μg in 1.5 μl) into the lateral ventricle and killed 24 hr later. Results are expressed as the mean ± S.E.M. together with the mean % of the estimates from concurrently assayed controls (100%). $n = 6$ for both groups. Significant differences from the controls are denoted by *$P < 0.05$, †$P < 0.01$ (Student's t test).

induced by local application of DA to this site [27].

In view of the number of discrepancies in the results of reported observations we are unable to conclude which, if any, of the sites discussed above are responsible for the EOS-induced decrease of DA turnover in the limbic region. In addition it is not possible to comment on the functional roles of either neuronal or glial GABA elements and their potential interaction with the DA neurone. However, a decreased DA turnover in the presence of elevated GABA concentrations would suggest a biochemically inverse relationship between these two neurotransmitter substances in certain brain regions.

Increased GABA concentrations were associated with certain regional (cortex, pons, mid-brain) increases in 5-HT utilisation as suggested by the elevated levels of the metabolite 5-HIAA. However it is difficult to establish if such an effect is directly related to increased GABA levels or may be the secondary result of, for example, a decreased food intake (associated with the akinetic state) which has been shown to increase whole brain 5-HT turnover [28]. Our results are in agreement with recent reports where intracerebroventricularly administered GABA was observed to enhance 5-HT turnover [29]. The exact site of action or mechanism of this proposed interaction between GABA and 5-HT or the possible involvement of other neurotransmitter systems is at present unknown, but would appear worthy of further investigation.

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