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GABAergic Mechanisms Mediating Rotational Behavior in Rats: Differences Between Dorsal and Ventral Striatum

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KAFETZOPOULOS, E *GABAergic mechanisms mediating rotational behavior in rats Differences between dorsal and ventral striatum* PHARMACOL BIOCHEM BEHAV 29(3)457–460, 1988 —Local injection of muscimol into the dorsal or ventral striatum of rats did not induce any rotational activity, but injection of picrotoxin into ventral striatal aspects induced an intense contralateral rotation Amphetamine pretreatment resulted in an ipsilateral rotation after muscimol injection into dorsal striatum and in a complex rotational pattern after ventral injection, characterized by an initial short-lasting ipsilateral rotation followed by a long-lasting contralateral rotation The contralateral rotation induced by picrotoxin was abolished by amphetamine pretreatment These results indicate a functional heterogeneity between dorsal and ventral striatal mechanisms mediating rotational behavior, and a different pattern of dopamine-GABA interactions between dorsal and ventral striatum

Striatum GABA Muscimol Picrotoxin Amphetamine Rotational behavior Rat

THERE is a large body of evidence suggesting that the GABAergic pathways originating from the striatum and projecting to the substantia nigra pars reticulata (SNr) and globus pallidus (GP) represent important striatal output systems, mediating the intrastriatal dopaminergic activity [4, 20, 21] One of the most extensively used tests of this striatal dopaminergic activity is rotational behavior following unilateral lesions of the nigrostriatal pathway in which direct acting dopamine (DA) receptor agonists cause contralateral rotation, presumably due to the development of DA receptors supersensitivity on the lesioned side [23] Lesion studies revealed that this DA-dependent rotational activity is primarily mediated by the striatofugal GABAergic efferents projecting to the SNr [3,9], although a role of the striatopallidal projection cannot be excluded [7,20]

The striatum of the rat, however, is not a homogeneous structure, as far as some conditioned [5, 11, 14, 27] or unconditioned behaviors [12,13] are concerned This differentiation seems to also include DA-dependent postural asymmetries possibly underlying rotational behavior Studies in the cat revealed a functional dissociation within the striatum, since DA injections into various striatal regions elicited contralateral head turning at some sites and ipsilateral at other sites [2,25] In the rat, although DA injections into various regions of the dorsal or ventral striatum did not induce asymmetry or rotational activity, they elicited a potentiation of all the contralaterally directed ongoing behaviors, which was more prominent after dorsal injections [8] In a recent study, however, Turski *et al* [22] reported that muscimol injection into dorsal or ventral striatum of rats induced contralateral or ipsilateral asymmetry respectively, suggesting a possible dorsal-ventral dichotomy between the striatal GABAergic mechanisms mediating postural asymmetry

In this latter study, however, the asymmetric postural deviations were assessed visually, immediately after the injections of muscimol and before the animals were immobilized for another test Under these conditions, some time-related effects of the stimulation of striatal GABAergic mechanisms on asymmetric locomotor behavior may be missed The purpose of the present study was to evaluate these effects by using automatic recording of the rotational behavior and to detect a possible functional dichotomy within the rat striatum, concerning asymmetric locomotor behaviors

METHOD

Animals

Twenty-four male Wistar rats weighing 260–330 g at the time of operation were used The animals were housed in cages of four animals each on a 12 hr light-dark cycle with free access to food and water

Surgery

Rats were anaesthetized with sodium pentobarbital (40 mg/kg, IP) and placed in a David Kopf stereotaxic frame Guide stainless steel cannulae (outer diameter 0 70 mm)

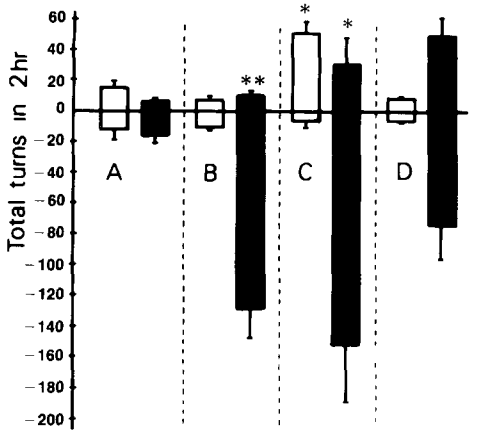

FIG 1 Rotational behavior expressed as total ipsilateral (positive) and contralateral (negative) turns in 2 hours after unilateral intrastriatal muscimol (A) or picrotoxin (B) injections in saline treated rats and muscimol (C) or picrotoxin (D) injections in amphetamine treated rats Open bars indicate dorsal striatal injections and close bars ventral intrastriatal injections *$p<0$ 05, **$p<0$ 01, paired Student's t-test, ipsilateral vs contralateral turns

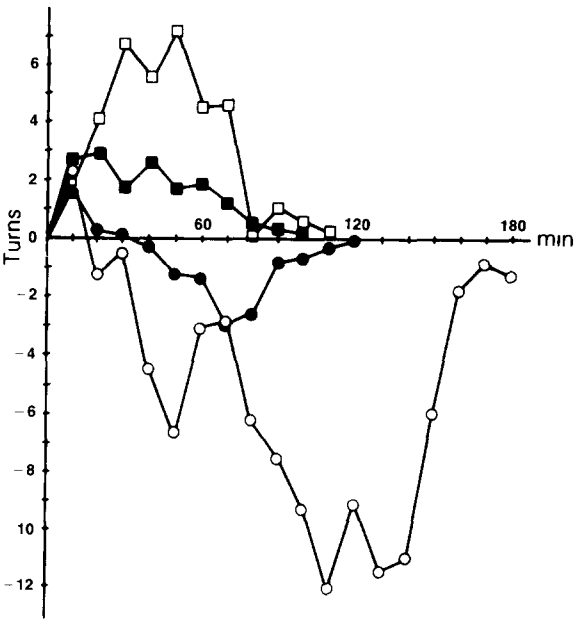

FIG 2 Rotational behavior produced by dorsal (squares) or ventral (cycles) intrastriatal injections of muscimol in saline (close symbols) or amphetamine (open symbols) pretreated rats Ordinates ipsilateral (positive) or contralateral (negative) turns per 10 min Abscissa minutes after the intrastriatal muscimol injections

were implanted bilaterally and attached by dental cement The cannulae plugged with a steel mandrel of the same length and aimed 1 mm above the injection coordinates AP 0 0 mm, 2 0–2 5 mm, V 4 and 6 mm [15]

Rotational Behavior

One week after the operation rats were tested for rotational activity after dorsal or ventral intrastriatal injections of muscimol 50 ng (Sigma) or picrotoxin 250 ng (Sigma) in 0 5 μl of saline, 30 min after IP saline or d-amphetamine 1 mg/kg (Sigma) pretreatment Rats were pretreated with saline or d-amphetamine and placed in electronic rotometers connected with a microcomputer When the 30 min elapsed, they were removed and injected intracerebrally with the appropriate drug by inserting an injection cannula (outer diameter 0 35 mm) connected via a polyethylene tube to a 5 μl Hamilton syringe driven by an injection pump (Harvard Apparatus) Injections were lasted 30 sec and the injection cannulae were left in place for a further 30 sec following each injection in order to minimize leakage of the drug up the cannula track Then the rats were returned to the rotometer cages and rotational behavior was recorded for 3 hours Non-cumulative 10 min interval printouts were taken on the system printer Each animal was injected twice into the same hemisphere with the same drug after saline and d-amphetamine pretreatment and the order of treatment was randomized Three days intervals were allowed between treatments

Histology

On completion of the experiments, a routine histological analysis was performed in order to verify the location of the tips of the injection cannulae Rats were decapitated after intracardial perfusion with formalin and the brains were stored in a formalin-sucrose solution Coronal sections, cut at 40 μ in a cryomicrotome, were stained with toluidin blue The exact cannula tip location was verified under microscopic examination and transferred visually on the corresponding plates of the Paxinos and Watson atlas (Fig 3)

Statistics

A two-way analysis of variance (ANOVA) was used to analyze total rotation scores expressed as differences between ipsilateral and contralateral total rotations after the first 2 hr period, with pretreatment (saline vs amphetamine) and injection (dorsal vs ventral) as factors Two different ANOVAs were performed, one with muscimol and one with picrotoxin data Student's t-tests were calculated between ipsilateral and contralateral rotations for all groups, with the level of $p<0$ 05 considered as critical for statistical significance of the observed differences in rotation preference Statistical analyses were performed on a microcomputer using the SPSS/PC statistical package

RESULTS

Intrastriatal muscimol injections in saline-pretreated rats was followed by a non-significant preference towards ipsilateral side after dorsal injections (Fig 1A) and a non-significant contralateral preference with an initial short lasting ipsilateral rotation after ventral injections (Fig 2) Amphetamine pretreatment potentiated this preference, so that it reached the statistical significance criterion (Fig 1C and 2) The ANOVA for muscimol rotation scores revealed a significant injection (dorsal vs ventral) effect, $F(1,44)=7$ 6, $p<0$ 01, a non-significant treatment (saline vs amphetamine) effect, $F(1,44)=1$ 43, $p=0$ 23, but a significant injection by treatment interaction, $F(1,44)=5$ 16, $p<0$ 03, indicating that the significant differences between dorsal and ventral injections were observed only after amphetamine treatment

Picrotoxin injection into ventral, but not dorsal, striatum induced an intense contralateral rotation (Fig. 1B), which was abolished by d-amphetamine pretreatment The ANOVA for the picrotoxin rotation scores revealed a significant injection effect, $F(1,44)=9$ 45, $p<0$ 01, and a significant

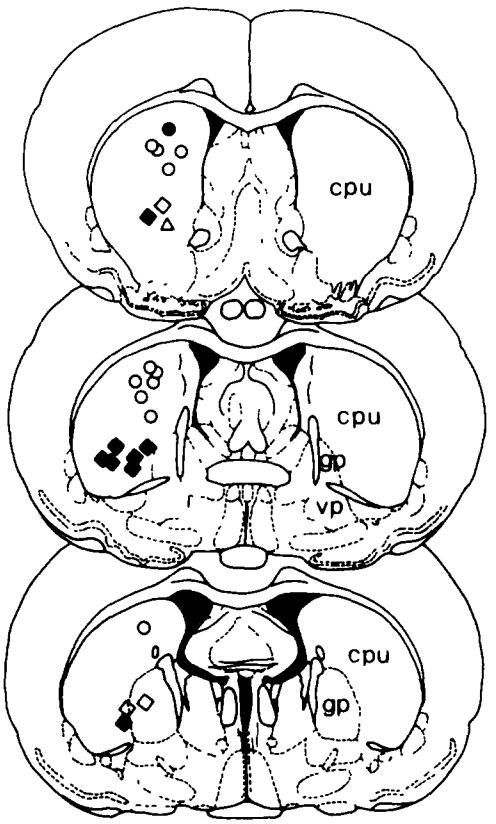

FIG 3 Cannulae tip location transferred visually on the same hemisphere Open symbols indicate ipsilateral rotation after muscimol injections in amphetamine pretreated rats, close symbols indicate biphasic ipsi-contralateral pattern Open triangle indicates no rotation Circles indicate cannulae tips aimed at dorsal striatum and diamonds indicate cannulae tips aimed at ventral striatum

treatment effect, $F(1,44)=5\ 43$, $p<0.03$, without any significant injection by treatment interaction, $F(1,44)=1\ 75$, $p=0\ 2$

DISCUSSION

According to our recent concepts of intrinsic striatal organization, GABA acts as a neurotransmitter within the striatum and is released either from terminals of GABAergic interneurons and/or from local axon collaterals of GABAergic strionigral and striopallidal output neurons [1,6] As derived from electrophysiological studies, the striatal output GABAergic neurons are in a state of strong and/or tonic inhibition induced by endogenous GABA [16, 17, 19, 26] Consequently, the GABAergic activity in the SNr and GP receiving projections from these striatal efferent GABAergic neurons may be relatively low [21]

In agreement with this hypothesis, in the present study the GABA agonist muscimol injected unilaterally into the striatum did not induce any statistically significant rotation, although intranigral or intrapallidal muscimol induces strong rotational activity [21] On the contrary, local injection of the GABA antagonist picrotoxin produced a marked contralateral rotation, observed however only after ventral injections

It has been suggested that the striatal GABAergic interneurons are under an inhibitory dopaminergic influence [6,18] Amphetamine pretreatment, therefore, acting probably by releasing DA into the striatum, would inhibit these interneurons, disinhibiting striatal output neurons Under the state of this disinhibition muscimol induced a significant rotational activity, while the picrotoxin-induced rotation was abolished

The complex rotational patterns, however, observed after intrastriatal muscimol injections in amphetamine pretreated rats indicate probably an underlying complexity of the GABAergic striatal mechanisms mediating asymmetric behaviors Not only dorsal and ventral injections induced opposite rotation, but ventral injections induced also a bidirectional rotational pattern, constantly reproduced in most of the rats These results are in agreement with previous studies concerning behavioral effects after bilateral GABA agonists injections or postural asymmetry after unilateral injections into the ventral striatum Muscimol and THIP injected bilaterally into the ventral striatum induced first a short-lasting catalepsy followed by a period of long-lasting dose-dependent locomotor stimulation Unilateral injections into the same region induced also an initial short-lasting ipsilateral postural asymmetry, followed by contralateral asymmetry, without rotational activity Injections of GABA agonists into other striatal areas did not induce any gross behavioral change [21] Since the initial ipsilateral postural asymmetry decreases considerably with increased distance from GP, it has been proposed that this effect may be due to infusion of muscimol into the GP, where GABA agonists induce ipsilateral postural asymmetry [20] The finding of the present study, however, that picrotoxin injection into the same area induced a marked contralateral rotation, while only minor asymmetry is observed after intrapallidal injection [20], indicates a rather direct action of GABA-related drugs on striatal than on pallidal GABAergic mechanisms

The same biphasic behavioral response, e g , initial sedation followed by enhanced locomotion, has been observed also after systemic injection of small doses of apomorphine [10], indicating a direct or indirect involvement of central dopaminergic mechanisms in the expression of this complex pattern Recently presented data suggests that this biphasic response may be due to the activation of different receptor subpopulations, since apomorphine-induced stimulation can be antagonized by D-1 as well as D-2 receptor blockage, while apomorphine sedation is antagonized by D-2 receptor blockage [24] In an attempt to relate DA receptor subtypes with GABAergic output systems, Herrera-Marschitz and Ungerstedt [7] provided evidence that the D-2 receptor agonist pergolide may induce inhibition of striopallidal GABAergic neurons, while the D-1/D-2 agonist apomorphine may inhibit GABAergic interneurons exerting disinhibition on the strionigral GABAergic projection

According to the above outlined scheme of DA-GABA interactions within the striatum, it seems reasonable to conclude that the ipsilateral and contralateral components of the rotational activity, as presented in this study, are due to the activation of the two distinct striatal output systems, the striopallidal and the strionigral GABAergic pathways Muscimol induced rotational activity after injections in both dorsal and ventral striatal aspects in amphetamine-treated rats, indicating a DA-GABA interaction in both areas The ipsilateral rotation observed after dorsal muscimol injections may be due to the inhibition of a dorsal striatum-SN GABAergic projection The finding that injection of muscimol or picrotoxin in this area in saline pretreated rats did not induce any rotational activity indicates that the GABAergic output

neurons in dorsal striatum maintain a functional equilibrium and stability which may be the result of collateral innervation Amphetamine pretreatment probably reduces the internal GABA pool, by inhibiting these output neurons, and consequently permits muscimol to exert its inhibitory effect on the strionigral neurons located into the ipsilateral dorsal striatum

On the contrary, in ventral striatum the GABAergic inhibition of the output neurons may be mediated by interneurons which inhibit both strionigral and striopallidal neurons After disinhibition of these output neurons by d-amphetamine, muscimol induces the ipsilateral component of the observed rotation by inhibiting the strionigral pathway and the contralateral component by inhibiting the striopallidal neurons

The findings of the present study support the above interpretation, but do not permit any definitive conclusion on this matter, considering the complexity of the internal striatal organization The rotating rat model, however, seems to be a very useful tool for the further investigation of the regional differences and the complex DA-GABA interactions within the striatum

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