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DIFFERENTIAL ROLE OF $GABA_A$ AND $GABA_B$ RECEPTORS IN TWO DISTINCT OUTPUT STATIONS OF THE RAT STRIATUM: STUDIES ON THE SUBSTANTIA NIGRA PARS RETICULATA AND THE GLOBUS PALLIDUS

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Abstract—The role of $GABA_A$ and $GABA_B$ receptors in the substantia nigra pars reticulata and the globus pallidus in turning behaviour of rats was studied. Unilateral injection of the $GABA_A$ receptor agonist muscimol (25 and 50 ng) into the substantia nigra pars reticulata elicited contralateral pivoting, namely tight head-to-tail turning marked by abnormal hindlimb backward stepping. This effect was $GABA_A$ receptor specific, since it was dose-dependent and prevented by co-administration of the $GABA_A$ receptor antagonist bicuculline (100 and 200 ng) which alone did not elicit turning behaviour. Unilateral injection of the $GABA_B$ receptor agonist baclofen (100 and 200 ng) into the substantia nigra pars reticulata also produced contralateral pivoting. This effect was $GABA_B$ receptor specific, since it was dose-dependent and inhibited by the $GABA_B$ receptor antagonist CGP 55845 (200 ng) which alone did not elicit turning behaviour. In contrast, unilateral injection of bicuculline (100 and 200 ng) into the globus pallidus produced contralateral circling, namely turning marked by normal stepping. This effect was $GABA_A$ receptor specific, since it was dose-dependent and prevented by muscimol (50 ng), which alone did not elicit turning behaviour. Unilateral injection of baclofen (100 and 200 ng) into the globus pallidus dose-dependently produced ipsilateral pivoting; this effect was inhibited by CGP 55845 (200 ng), which alone did not elicit turning behaviour. The present study demonstrates that $GABA_A$ and $GABA_B$ receptors in the globus pallidus and the substantina nigra pars reticulata play differential roles in the production of turning behaviour. This study underlines the notion that the two types of turning, namely pivoting and circling, are valid tools to map out the information flow across the basal ganglia.

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Abbreviations: ANOVA, analysis of variance; EP, entopeduncular nucleus; GP, globus pallidus; IC, inferior colliculus; mdT, mediodorsal thalamus; Nacc, nucleus accumbens; PPN, pedunculopontine tegmental nucleus; SNR, substantia nigra pars reticulata; STN, subthalamic nucleus; VLS, ventrolateral striatum; vmT, ventromedial thalamus; VP, ventral pallidum.

Key words: turning behaviour, ventrolateral striatum, mediodorsal thalamus, pedunculopontine tegmental nucleus, ventral pallidum, nucleus accumbens shell.

The basal ganglia are implicated in numerous sensorimotor, cognitive and motivational processes. Moreover, the basal ganglia are known to be involved in the etiology of various neuropsychiatric disorders, including addiction, Parkinson's disease, ballismus, Huntington's chorea, Gilles de la Tourette's syndrome, schizophrenia, attention deficit hyperactivity disorder, depression and the side-effects of both antipsychotic and antiparkinson agents. The macrocircuitry of the basal ganglia is highly complex: the basal ganglia are now recognized as a series of parallel, functionally segregated circuits that show interactions at various levels (for review, see Groenewegen et al., 1999). Although anatomical and electrophysiological studies have given insight into the function of the various parallel circuits, the flow of information is not fully understood and is still a matter of discussion. Previously, we have shown that two types of turning, namely circling and pivoting, are excellent tools for mapping the flow of information across the basal ganglia (Koshikawa, 1994; Saigusa et al., 1995; Kitamura et al., 1999, 2001; Ikeda et al., 2004, 2009a,b; Moribe et al., 2005): as elaborated below, studies using these readout parameters have shown that there are distinct pathways conveying basal ganglia outflow and demonstrate that each type of turning is funnelled by its own characteristic pathway. During circling, the rat steps forward with both hindlimbs and the steps of the outer forelimb frequently cross those of the inner forelimb resulting in a much larger turning circle; during pivoting, the rat steps backward with one hindlimb while pivoting on the other hindlimb so that the turning circle is less than the body length (Saigusa et al., 1995). Both circling and pivoting need a drive to move forward, requiring the involvement of the nucleus accumbens (Nacc; Swanson et al., 1997; Ikemoto, 2002). More importantly, circling is marked by a characteristic type of forelimb stepping, requiring the involvement of the Nacc (Cools and Jongen-Relo, 1991) in contrast to pivoting that is marked by a characteristic type of hindlimb stepping, requiring the involvement of the neostriatum (Cools and Jongen-Relo, 1991). As mentioned below, circling is funnelled via a pathway that does not necessarily involve the neostriatum in contrast to pivoting that does involve the neostriatum. For these reasons, both circling and pivoting are chosen as tools for mapping

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more in detail the flow of information across the basal ganglia.

It is already known that contralateral pivoting that can be elicited by simultaneous stimulation of dopamine D_1 and D_2 receptors unilaterally in the shell of the Nacc (Saigusa et al., 1995; Kitamura et al., 1999, 2001) requires an intact, ipsilateral ventrolateral striatum (VLS; Saigusa et al., 1995). This pivoting is also seen after stimulation of $GABA_A$ receptors in the pedunculopontine tegmental nucleus (PPN; Ikeda et al., 2004). On the basis of these and related data, we have suggested that the anatomical circuit that mediates this pivoting is a polysynaptic pathway that funnels the relevant information from the shell of the Nacc to the PPN *via* the accumbens-nigro-striato-nigro-PPN pathway (Ikeda et al., 2004). Because the role of the substantia nigra pars reticulata (SNR) in pivoting is not yet known, we decided to study the role of $GABA_A$ and $GABA_B$ receptors in eliciting pivoting from the SNR. It is already known that one can elicit contralateral turning from the unilateral SNR (Olpe et al., 1977; Scheel-Krüger et al., 1977; Waddington, 1977; Martin et al., 1978; Olianas et al., 1978; Waddington and Cross, 1978; Arnt and Scheel-Krüger, 1979; Reavill et al., 1979; Kozlowski and Marshall, 1980; Kilpatrick, 1986), but the type of turning is unknown. Although both the $GABA_A$ agonist muscimol and the $GABA_B$ agonist baclofen have been found to be effective when applied into the SNR (see above), hard evidence in favour of the factual involvement of $GABA_A$ and $GABA_B$ receptors in the SNR is not yet available.

In contrast to contralateral pivoting that can be elicited by stimulation of dopaminergic receptors in the unilateral shell of the Nacc, contralateral circling can be elicited by stimulation of acetylcholine receptors in the unilateral shell of the Nacc (Saigusa et al., 1995; Kitamura et al., 1999). This circling is funnelled via a circuit that differs from that involved in pivoting (Kitamura et al., 1999, 2001; Akiyama et al., 2004; Ikeda et al., 2004, 2007, 2009a,b; Moribe et al., 2005): in fact, it has been shown that the ventral pallidum (VP), one of the main output structures of the Nacc (for review, see Groenewegen et al., 1999), and the mediodorsal thalamus (mdT) are essential for the display of circling elicited from the Nacc (Kitamura et al., 2001; Ikeda et al., 2009b).

The globus pallidus (GP) is placed at the centre of the basal ganglia connections. In the present context, it is important to recall that it receives afferents of various nuclei that form part of the above-mentioned accumbens-nigro-striato-nigro-PPN pathway that is involved in pivoting and the accumbens-pallido-thalamic circuit that is involved in circling (for review, see Kita, 2007). For instance, the GP receives afferents from the PPN that is involved in pivoting as well as afferents from the VLS that is involved in both pivoting and circling (Koshikawa et al., 1990, 1996; Saigusa et al., 1993, 1995; Kitamura et al., 1999; for review, see Kita, 2007). Given the central position of the GP in the basal ganglia, we hypothesized that the GP is involved in both pivoting and circling. Until now, it is known that $GABA_B$ receptors in the GP are involved in the display of turning (Chen et al., 2002): it remains to be investigated which type of turning is elicited from the GP. Because the GP contains not only $GABA_B$ receptors but also $GABA_A$ receptors (for review, see Boyes and Bolam, 2007), the role of $GABA_A$ receptors has to be studied also.

To elucidate these issues, we investigated the role of $GABA_A$ and $GABA_B$ receptors in the SNR and the GP in the production of pivoting and circling, respectively. The present study shows that $GABA_A$ and $GABA_B$ receptors in the SNR and GP are differentially involved in the display of circling and pivoting. It provides evidence that both $GABA_A$ and $GABA_B$ receptors in the SNR and $GABA_B$ receptors in the GP are involved in the information flow that funnels pivoting; these receptors play no role in the information flow that funnels circling. In contrast, only $GABA_A$ receptors, but not $GABA_B$ receptors, in the GP are involved in the information flow that funnels circling.

EXPERIMENTAL PROCEDURES

Animals and surgery

Male Wistar rats (Saitama Experimental Animals Supply Co. Ltd., Saitama, Japan) weighing 190–210 g at the time of surgery were housed in cages ($27\times45\times20$ cm^3) that were kept at constant room temperature (23 ± 2 °C) and relative humidity (55 ± 5%) under a 12 h light/dark cycle (lights on at 0700 h), with free access to food and water.

For stereotactic implantation of cannulae, rats were anaesthetized with sodium pentobarbitone (Dainippon Sumitomo Pharma, Osaka, Japan; 50 mg/kg i.p.) and placed in a stereotactic apparatus (Narishige, Tokyo, Japan). A guide cannula (0.5 mm o.d., 0.3 mm i.d., 6.0 mm length for SNR and 4.0 mm length for GP) was planted into the SNR (A 3.7 mm, V 2.0 mm, L 2.8 mm from the interaural line) or the GP (A 8.0 mm, V 3.5 mm, L 3.0 mm from the interaural line), according to the atlas of Paxinos and Watson (1998). The cannulae were secured to the skull with stainless screws and dental acrylic cement. For the GP, cannulae were angled 10° from the midsagittal plane to avoid the ventricular system. Damage to the target site was minimized by implanting tips of the guide cannulae 2.0 mm (SNR) and 3.0 mm (GP) above the desired injection site. A wire stylet was placed in the guide cannula to prevent occlusion. The rats were then allowed to recover from surgery for a minimum of 5 days.

These experiments were performed in accordance with Institutional Guidelines for the Care and Use of Experimental Animals that are in compliance with the UK Animals (Scientific Procedures) Act, 1986. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Intracerebral microinjection and drugs

The drugs used were: muscimol (5-aminomethyl-3-hydroxyisoxazole; Sigma, St. Louis, MO, USA), a $GABA_A$ receptor agonist; (−)-bicuculline methbromide (Sigma), a $GABA_A$ receptor antagonist; *R*(+)-baclofen hydrochloride (*R*(+)-β-(aminomethyl)-4-chlorobenzenepropanoic acid hydrochloride; Sigma), a $GABA_B$ receptor agonist; CGP 55845 ((2S)-3-[[(1S)-1-(3,4-dichlorophenyl)ethyl]-amino-2-hydroxypropyl]phenylmethyl phosphinic acid; Tocris, Ellisvile, MO, USA), a $GABA_B$ receptor antagonist. All drugs were dissolved in saline (0.9% w/v NaCl solution) immediately before use. For unilateral intracerebral microinjection, rats were held manually while the stylets were removed and the injection needles (0.22 mm) lowered through guide cannulae so that they protruded beyond the tip by 2.0 mm for SNR and by 3.0 mm for GP. Needles were connected to Hamilton syringes and drugs were slowly given by hand in a volume of 0.2 μl over 20 s, after which needles were