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Serotonin_{2C} receptors in the ventral pallidum regulate motor function in rats

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Abstract

The ventral pallidum is a limbic brain region that regulates motor function. This region is extensively innervated by serotonergic neurons from the dorsal raphe nucleus. Serotonergic receptors, including the 5-HT_{2C} receptor subtype, are located in the ventral pallidum. However, little is known regarding the behavioral consequences of serotonergic transmission in the ventral pallidum, and the role of 5-HT_{2C} receptors has not been studied. To address this paucity, we measured the motoric consequences of injections of 0.33–10 ng of the 5-HT_{2C} receptor agonist MK 212 into the ventral pallidum of adult male Sprague–Dawley rats. We determined that locomotor activity was attenuated by 6.6 ng MK 212, and rearing was attenuated by both 1 and 6.6 ng. The motor suppressant effects of MK 212 were lost at the higher dose of 10 ng, likely reflecting a loss of selectivity of this ligand. These findings indicate negative regulation of motor function by 5-HT_{2C} receptors in the ventral pallidum.

Keywords

5-HT_{2C} receptor; MK 212; motor activity; serotonin; ventral pallidum

Introduction

The limbic system of the brain regulates complex reward-motivated motor behaviors. The limbic pallidum or ‘ventral pallidum’ is known to influence several aspects of these behaviors including motor activity, reward-mediation, and cognitive function. The ventral pallidum is innervated by several other limbic structures, for example the ventral tegmental area, nucleus accumbens, basolateral amygdala, raphe nucleus, medial subthalamic nucleus,

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Conflicts of interest

For the remaining authors there are no conflicts of interest.

and frontal cortex. Neurons in the ventral pallidum are primarily GABAergic projection neurons which terminate in all of the above-listed structures, with a subpopulation of cholinergic neurons that are largely corticopetal [1]. Pallidal neurons of both phenotypes are densely innervated by serotonergic terminals of the dorsal raphe nucleus projections [2]. Understanding the function of the receptors associated with these serotonin (5-HT)-containing terminals will help decipher the role that the ventral pallidum has in behaviors that are under limbic control. We have demonstrated that 5-HT_{1A} [3] and 5-HT_{2A/C} receptors [4] regulate neuronal firing in the ventral pallidum. The ability of systemically administered 2,5-dimethoxy-4-iodoamphetamine (DOI; a 5-HT_{2A/C} receptor agonist) to alter firing is enhanced in rats that show methamphetamine-induced motor sensitization (i.e. an enhancement in motoric activity resulting from repeated administration of the stimulant) [4]. However, whether 5-HT_{2C} receptors within the ventral pallidum directly regulate motor activity remained unknown. The current report addressed this knowledge gap by administering the 5-HT_{2C} receptor agonist MK 212 into the ventral pallidum and recording changes in motor activity.

Materials and methods

Male Sprague–Dawley rats ($n = 15$; Harlan Laboratories, Indianapolis, Indiana, USA) weighing 225–250 g upon arrival were housed in pairs, handled daily, and allowed to acclimate to the vivarium for at least 5 days before experimentation. Food and water were provided *ad libitum* throughout the study. All procedures were performed in accordance with the ‘*Guide for the Care and Use of Laboratory Animals*’ (National Research Council, Washington, District of Columbia, USA) with protocols approved by the Rush University Institutional Animal Care and Use Committee.

Rats were bilaterally implanted with guide cannula (23 G, 5mm wide, 16mm long) overlying the ventral pallidum per our prior procedures [5]. Approximately 1 week after surgery, rats were habituated to motor activity monitoring chambers (automated using two stacked banks of photobeams; Accuscan Instruments Inc., Columbus, Ohio, USA) for 60 min before intraventral pallidal injections and motor testing. The intraventral pallidal infusions were administered to unrestrained rats in a separate holding chamber using a Cambridge Instruments (Somerville, Massachusetts, USA) injection pump connected to PE10 tubing and 30G injectors that were inserted into the guide cannula. The injector tips extended 1mm below the cannula and infusions were given at a rate of 0.1 $\mu\text{l}/\text{min}$ for a total volume of 0.5 $\mu\text{l}/\text{side}$, after which the injectors were left in place for 1min before removal. The 5-HT_{2C} receptor agonist MK 212 was administered bilaterally into the ventral pallidum at 0.33, 1.0, 6.6, and 10.0 ng in a pseudorandomized order. MK 212 has nM affinity for the 5-HT_{2C} receptor with more than a 16-fold lower affinity for other receptors [6]. Rats were administered two to four doses including vehicle or sham (the latter referring to insertion of injectors with no fluid injected), with at least 3 days interposed between each administration. After an intraventral pallidal infusion, rats were immediately placed back into motor boxes. Horizontal activity, indicative of locomotion, and vertical activity, indicative of rearing, were recorded. Assessment data were totaled for 30min of testing and analyzed using a one-way analysis of variance with Newman–Keuls post-hoc analysis; $\alpha = 0.05$. Placement of the

injector tips were verified *post mortem* in brain sections by two observers blinded to the treatment protocol.

Results

Histological examinations verified that the intracerebral injections occurred throughout both the dorsal lateral and ventral medial subregions of the ventral pallidum (e.g. see Dallimore *et al.* [5]). Administration of the 5-HT_{2C} receptor agonist MK 212 into either subregion reduced both horizontal and vertical activity. Horizontal activity was decreased by 6.6 ng MK 212 (Fig. 1a). Vertical activity was decreased by 1.0 and 6.6 ng MK 212 (Fig. 1b). The motor suppressant effects did not occur with 10 ng MK 212 (Fig. 1a and b).

Discussion

Neurotransmission of GABA, glutamate, dopamine, and opioids within the ventral pallidum have long been known to regulate motor behavior (for review, see Napier [7]). The current report expands this knowledge base by showing that 5-HT transmission, at least by activation of 5-HT_{2C} receptors in the ventral pallidum, is sufficient to impact normal exploratory motor activity.

Ventral pallidal control over motor behaviors is complex (see Napier [7]) and the motoric profile seen with intrapallidal treatments depends on the engaged transmitter system and the dose of the tested ligand. To the best of our knowledge, agonist-induced reductions in motor activity following injections into the ventral pallidum have only been reported for low concentrations of the GABA_A agonist, muscimol (2–5 ng/side, higher concentrations increase locomotion; see Napier [7]), and the D2/D3 agonist, quinpirole [8]. The only drug tested thus far that enhances 5-HT transmission is amphetamine, and amphetamine administered in the ventral pallidum increases locomotion [9,10]. Thus, the ability of the 5-HT_{2C} receptor agonist MK 212 to reduce motoric outcomes was unexpected.

Immunohistochemical studies indicate that brain 5-HT_{2C} receptors are most densely localized within GABAergic neurons in the nucleus accumbens [11]. 5-HT_{2C} receptors within the ventral pallidum [12] may also be located on GABAergic neurons [13]. The canonical pathway engaged by the 5-HT_{2C} receptor (i.e. G_q/PLC pathway) is expected to facilitate neuronal depolarization and spiking. This cellular mechanism would predict that MK 212 administered in the ventral pallidum would enhance motor function, as seen for intrapallidal injections of the stimulant amphetamine [9,10], and the glutamatergic agonist, AMPA [14], both of which increase ventral pallidal neuronal spiking [15,16]. However, given motor reductions observed here, we propose the following scenarios: (i) Different GABAergic projection neurons differentially express 5-HT_{2C} receptors. For example, if 5-HT_{2C} receptor-expressing GABAergic projections preferentially terminated within the subthalamic nucleus, this would reduce the subthalamic excitatory drive to the pedunclopontine nucleus [17], and in so doing, reduce motor function. (ii) Noncanonical 5-HT_{2C} receptor signaling pathways (e.g. G_{i/o} and phospholipase A2 [18]) may be engaged by MK 212 in the ventral pallidum so that 5-HT_{2C} receptor activation reduces GABAergic

outflow and, as seen with injections in the ventral pallidum with low doses of muscimol, reduces motor function.

Most studies published to date have focused on ventral pallidal-regulation of horizontal locomotor activity. Here we show that this region regulates both horizontal and vertical components of spontaneous/normal exploratory behavior and that vertical activity (i.e. rearing) was more sensitive to 5-HT_{2C} receptor stimulation than was horizontal activity (i.e. locomotion). We have also consistently found that the motoric profile evoked by methamphetamine administration encompasses enhanced rearing behaviors [19–21], and this may reflect a functional upregulation of 5-HT_{2A/C} receptors as in methamphetamine-sensitized rats, the potency of the 5-HT_{2A/C} receptor agonist, DOI, to augment neuronal activity in the ventral pallidum is increased [4]. Moreover, we have previously observed that in rats trained to self-administer methamphetamine, a putative 5-HT_{2C} receptor inverse agonist, SB 206553, also attenuates rearing behavior [21]. These findings indicate that rearing behavior needs to be included in motoric assessments of ventral pallidal function.

The motoric effects of intrapallidal MK 212 were bimodal, wherein the ability of 1 and 6.6 ng MK 212 to reduce motor function was lost at 10.0 ng. This outcome may reflect a recruitment of additional 5-HT₂ receptor subtypes by the higher dose, potentially the 5-HT_{2A} receptor. When systemically administered, 5-HT_{2A} and 5-HT_{2C} receptor ligands often have oppositional effects on behavioral outcomes, including locomotor activity. For example, systemic administration of 5-HT_{2A} and 5-HT_{2C} receptor antagonists decreases and increases, respectively, cocaine-evoked hyperactivity in rats [22]. The effects of 5-HT_{2A} and 5-HT_{2C} receptors within the ventral pallidum may similarly have oppositional effects on behavioral outcomes. Thus, the higher doses of intrapallidal MK 212 may have activated 5-HT_{2A} receptors and caused physiological antagonism of 5-HT_{2C} receptors, thereby masking 5-HT_{2C} receptor-dependent suppression of motor activity.

It is also of interest that the effective intraventral pallidal MK 212 doses were comparatively small. Bilateral administration of 0.05–0.5 µg MK 212 into the nucleus accumbens is not sufficient to alter exploratory motor activity [23], whereas doses as low as 1.0 ng in the ventral pallidum were sufficient to reduce behavior. This dramatic leftward shift in dose–response relationship between the ventral pallidum and nucleus accumbens may reflect the fact that the ventral pallidum is downstream of the accumbens in brain systems that regulate motor activity. Moreover, the ventral pallidum contains ~7-fold higher levels of 5-HT than striatal regions [24]; therefore, the differences in effective MK 212 doses between the accumbens and ventral pallidum may reflect differential sensitivity of these two regions to changes in serotonergic processes. Serotonergic regulation of motor activity within the ventral pallidum may also be independent of the nucleus accumbens, a phenomenon known to occur for neuronal responses to D2 dopamine receptor activation in the ventral pallidum [25].

Conclusion

The current report is the first to assess the effects of administering a 5-HT_{2C} receptor agonist within the ventral pallidum on motor activity. We provide evidence that 5-HT_{2C} receptors in

the ventral pallidum provide inhibitory regulation of motor activity and do so at lower doses than those reported to produce similar behavioral effects from the nucleus accumbens [23].

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Fig. 1.

Administration of the 5-HT_{2C} receptor agonist MK 212 into the ventral pallidum decreased exploratory motor activity. (a) Horizontal activity was reduced at 6.6 ng, and the effect was lost at 10 ng [$F_{(4,39)} = 4.677$]. (b) Vertical activity was reduced by 1 and 6.6 ng, and the effect was lost at 10 ng [$F_{(4,39)} = 3.230$]. Shown are significance from shams, determined by Newman-Keuls post-hoc analysis; * $P < 0.05$.