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Research Report

Chemogenetic inhibition of cells in the paramedian midbrain tegmentum increases locomotor activity in rats

*David Wirtshafter**, *Thomas R. Stratford*

Laboratory of Integrative Neuroscience, Department of Psychology (m/c 285), University of Illinois at Chicago, 1007 West Harrison Street, Chicago, IL 60607-7137, USA

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ABSTRACT

Pronounced hyperactivity can be produced by lesions or pharmacological inhibition of cells in the median raphe nucleus (MR) located in the paramedian midbrain tegmentum. In the current study we examined whether a similar effect can be seen after chemogenetic inhibition of cells in this region using the DREADD (Designer Receptors Exclusively Activated by Designer Drugs) approach. We found that the DREADD ligand clozapine-N-oxide (CNO) increased locomotor activity in animals expressing the inhibitory DREADD hM4Di, but not those injected with a control virus in the MR. The effect was of rapid onset and short duration and persisted for at least four months after virus injections. Histological examination of the brains indicated that labeled fibers followed the known projection patterns of the MR to a variety of forebrain and midbrain structures. These findings confirm the role of the MR region in the control of locomotion and suggest that the DREADD technique may be a useful approach to the study of the functional architecture of this complex area. Methodological and interpretive aspects of DREADD studies are discussed.

1. Introduction

The median raphe nucleus (MR), also known as the nucleus centralis superior, is a structure lying in the paramedian portion of the caudal mesencephalic tegmentum that appears to exert a remarkably powerful influence on a variety of behaviors. Especially pronounced effects are seen on measures of locomotor activity. Thus, marked increases in locomotion in a variety of settings are seen after electrolytic or excitotoxic lesions of the MR (Asin and Fibiger, 1983; Geyer et al., 1976; Lorens et al., 1971; Wirtshafter and Asin, 1982) or after inhibition of MR cells produced by local injections of $GABA_A$ or $GABA_B$ agonists or of excitatory amino acid antagonists (Wirtshafter et al., 1987, 1989, 1993). These effects are strikingly resistant to blockade by systemic administration of D_2 dopamine antagonists (Shim et al., 2014; Wirtshafter et al., 1988), suggesting that they are not secondary to alterations in dopamine release. In like fashion, increases in food intake

*Corresponding author. Fax: +1 312 413 4122.
E-mail address: davew@uic.edu (D. Wirtshafter).

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can also be produced by intra-MR injections of GABA agonists and glutamate antagonists (Wirtshafter, 2000, 2011; Wirtshafter and Trifunovic, 1988). These effects on both activity and feeding are anatomically specific to the MR, and much smaller responses are seen with drug injections rostral, caudal, dorsal or lateral to the nucleus (Klitenick and Wirtshafter, 1988; Wirtshafter et al., 1989, 1993; Wirtshafter and Klitenick, 1990).

Although the MR is best known as a major source of serotonergic projections to a number of forebrain sites including the hippocampus (Moore, 1981), the majority of MR cells utilize transmitters other than serotonin (Leger and Wiklund, 1981). For example, large numbers of neurons expressing various GABA and glutamate markers are found in the MR, and even serotonergic MR cells may colocalize other transmitters (Mintz and Scott, 2006;Mugnaini and Oertel, 1985). All MR projections studied to date contain a nonserotonergic component, although the relative proportions of serotonergic and nonserotonergic cells may well differ in various pathways (Aznar et al., 2004;Aznar and Knudsen, 2002;Szonyi et al., 2015). In line with these anatomical data, a substantial body of evidence indicates that serotonin plays, at most, a minor role in the effects produced by MR manipulations. For example, selective destruction of serotonergic cells does not reproduce the hyperactivity seen after nonselective lesions (Asin and Fibiger, 1983;Geyer et al., 1980;Lorens, 1978) and intra-MR injections of serotonin autoreceptor agonists produce much smaller effects on locomotion than do injections of the $GABA_A$ agonist muscimol, even at doses which produce similar effects on hippocampal serotonin release (Shim et al., 1997). These results all suggest an important behavioral function for transmitters other that serotonin in MR function.

Dissecting out the functional role of various chemically or connectionally defined populations of MR cells is a challenging task and one that would appear likely to be facilitated by the recently developed DREADD (Designer Receptors Exclusively Activated by Designer Drugs) technique (Urban and Roth, 2015;Wess et al., 2013). In one version of this approach, a virus is used to infect cells with a gene coding for a modified form of the inhibitory m4 acetylcholine receptor (hM4Di). This mutated receptor is insensitive to acetylcholine, but can instead be activated by the relatively inert agent clozapine-N-oxide (CNO). Thus, systemic administration of CNO will selectively inhibit neurons which express the DREADD construct. The effects of CNO can even be restricted to specific genetically defined populations of cells by injecting vectors for Cre dependent DREADDs into animals genetically modified to express Cre recombinase under the control of specific promoters (Shapiro et al., 2012). The hM4D receptors are expressed not only in the cell body, but also in axon terminals, so it may even be possible to presynaptically inhibit transmitter release in terminal fields by local injections of CNO (Mahler et al., 2014).

Although the DREADD approach would appear to hold substantial promise for the study of the MR, there is currently no direct evidence that it will work in this system. The inhibitory DREADD method has been employed in a relatively small number of experiments in rats, as compared to mice, and appears to have obtained a reputation for being difficult to use successfully at the behavioral level in rats. In contrast, although a few behavioral studies of the MR have been conducted in mice (Martin and van den Buuse, 2008; Pezzato et al., 2015) the overwhelming majority of such experiments have been carried out in rats, a choice which is reasonable given the small size of this nucleus. In view of these considerations, we attempt in the current experiment to examine whether hM4Di mediated effects on cells in the paramedian tegmentum is able to produce alterations in locomotor activity similar to those seen after conventional pharmacological inhibition of MR cells. We examined the locomotor responses CNO both in rats transfected with non-Cre dependent hM4Di and in animals injected with a control virus which did not code for the DREADD construct. In order to examine how long responsiveness to CNO persisted following viral injections, we studied the response to CNO both in experiments beginning 20 days following viral injections and again four months later. We also examined whether CNO injections in DREADD-expressing animals would increase food intake, as do intra-MR injections of a variety of inhibitory drugs.

2. Results

2.1. *Experiment 1. Effects of CNO in animals with DREADD virus injections into the MR*

2.1.1. *Effects on locomotor activity in rats with DREADD virus injections*

Locomotor activity in response to injections of CNO at doses of 2.5 or 10 mg/kg, or its vehicle, was measured between 20 and 24 days following surgery and responses to the 10 mg/kg dose and vehicle were reassessed 140–142 days following surgery. Locomotor activity counts during the initial set of tests are shown in the upper panel of Fig. 1. Examination of the figure shows that CNO tended to produce a dose dependent increase in locomotion. This impression was supported by 2-way (dose × time) repeated measures ANOVA conducted on the 10 12 min long bins which followed drug treatments. This analysis indicated a significant effect of dose ($F(2,10)=10.06$, $p<0.005$) and *post-hoc* comparisons using the Fisher-LSD approach indicated that both doses of CNO increased overall activity with respect to vehicle, and that the overall response was significantly higher at the 10.0 mg/kg dose than the 2.5 mg/kg dose ($p<0.03$ in all cases). Examination of Fig. 1 indicates that the response occurred with very short latency, being maximal within the first 12–24 min, and then decayed rapidly. The ANOVA indicated that the dose × time interaction was significant ($F(18,90)=2.37$, $p<0.005$), and *post-hoc* contrasts indicated that the dose effect was significant across the first 4 time bins ($p<0.053$ for bin 1, $p<0.05$ for bins 2–4), but not throughout the remainder of the session.

The lower panel of Fig. 1 shows the response to vehicle and 10 mg/kg CNO in the same animals tested approximately 120 days after the test described above. It can be seen that CNO again induced a short latency increase in locomotion which decayed to control levels over a relatively brief period. ANOVA indicated a significant effect of CNO ($F(1,5)=9.40$, $p<0.05$) and of the CNO × time interaction ($F(9,45)=2.60$,