Neuroscience 131 (2005) 475–479

EFFECTS OF INJECTIONS OF 8-HYDROXY-2-(DI-*N*-PROPYLAMINO)TETRALIN OR MUSCIMOL IN THE MEDIAN RAPHE NUCLEUS ON C-*FOS* mRNA IN THE RAT BRAIN

D. FUNK,[a*] Z. LI,[a] P. J. FLETCHER[a,c,d] AND A. D. LÊ[a,b,c]

[a]*Department of Neuroscience, Center for Addiction and Mental Health, Toronto, Ontario, Canada M5S 2S1*

[b]*Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 1A8*

[c]*Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada M5T 1R8*

[d]*Department of Psychology, University of Toronto, Toronto, Ontario, Canada M5S 1A8*

Abstract—Inhibition of the median raphe nucleus (MRN) by the local injection of 5-HT_{1A} or $GABA_A$ receptor agonists produces strong activational effects on feeding, drinking and locomotor activity. Using an animal model of relapse, we have shown that intra-MRN injection of the 5-HT_{1A} autoreceptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) reinstates alcohol seeking in rats. The circuitry underlying the behavioral effects of intra-MRN injection of these drugs is not known. In order to identify the brain areas that may be involved, we measured levels of mRNA of the immediate early gene c-*fos* in discrete nuclei of the rat brain following intra-MRN infusions of these drugs. Male Wistar rats received intra-MRN infusions of 8-OH-DPAT (1 μg), muscimol (25 ng) or saline vehicle immediately prior to placement in locomotor activity chambers. Thirty minutes later, they were decapitated, and their brains processed for *in situ* hybridization of c-*fos* mRNA. In agreement with previous reports, injections of 8-OH-DPAT or muscimol into the MRN resulted in large increases in locomotor activity. Intra-MRN injections of these drugs increased c-*fos* in a number of brain nuclei previously shown to be involved in the rewarding effects of drugs of abuse in a regionally specific manner. Both drugs significantly increased the expression of c-*fos* mRNA in the medial frontal cortex, nucleus accumbens, lateral septum, dorsal bed nucleus of the stria terminalis and ventral tegmental area. In the ventral hippocampus, only 8-OH-DPAT increased c-*fos,* while in the basolateral nucleus of the amygdala and locus coeruleus, it was increased only by muscimol. These results are discussed in terms of the projections of the MRN and the pathways involved in relapse to alcohol and drug seeking. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: 5-hydroxytryptamine, GABA, *in situ* hybridization, locomotor activity, reinstatement, relapse.

*Corresponding author. Tel: +1-416-535-8501x6751; fax: +1-416-595-6922.
E-mail address: douglas_funk@camh.net (D. Funk).

Abbreviations: BLA, basolateral amygdala; BST, bed nucleus of the stria terminalis; LC, locus coeruleus; LS, lateral septum; MFC, medial frontal cortex; MRN, median raphe nucleus; MS, medial septum; NAc, nucleus accumbens; VTA, ventral tegmental area; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin.

0306-4522/05$30.00+0.00 © 2005 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2004.11.026

Acute inhibition of the median raphe nucleus (MRN) by the local microinjection of drugs has been reported to exert potent activational effects on a number of behaviors, including feeding, water and alcohol drinking and locomotor activity (Sainati and Lorens, 1982; Fink and Morgenstern, 1986; Wirtshafter et al., 1987; Klitenick and Wirtshafter, 1988; Tomkins et al., 1994; Wirtshafter, 2001). Using a rat model of drug relapse (de Wit and Stewart, 1981), we have shown that pathways originating in the MRN play an important role in relapse to alcohol seeking. We found that intra-MRN infusions of the 5-HT_{1A} autoreceptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT; that would inhibit the firing of MRN 5-HT neurons) or the stress-related hormone corticotropin-releasing factor, reinstated extinguished operant responding for alcohol (Lê et al., 2002). More recently, we have become interested in extending these studies to the role of the inhibitory amino acid transmitter GABA in the MRN in relapse to alcohol.

The goal of the present study was to identify the brain areas that may be involved in the behavioral effects of these drugs. We examined the effects of MRN injections of the 5-HT_{1A} receptor agonist 8-OH-DPAT or the $GABA_A$ receptor agonist muscimol on the expression of c-*fos* mRNA in discrete nuclei of the rat brain. C-*fos* is an immediate early gene that has been used to study patterns of neuronal activation evoked by physiological, pharmacological and environmental manipulations (Curran and Morgan, 1995; Kovacs, 1998). We have used this technique to explore the involvement of the MRN in footshock-induced relapse to alcohol seeking (Funk et al., 2003). In the present study, we examined c-*fos* expression in brain areas innervated by the MRN (septum, hippocampus; Vertes et al., 1999) as well as other brain areas involved in reinstatement of drug seeking (medial frontal cortex (MFC), nucleus accumbens (NAC), bed nucleus of the stria terminalis (BST), amygdala; Shaham et al., 2000; Shalev et al., 2002), and alcohol reward (ventral tegmental area, VTA; Weiss and Porrino, 2002).

EXPERIMENTAL PROCEDURES

Twenty-four male Wistar rats (Charles River, Montreal, Quebec, Canada) weighing 300–350 g were maintained at 22 °C (light phase 07:00 h–19:00 h) with free access to food and water. Guide cannulae (24 gauge; Plastics One, Roanoke, VA, USA) were implanted at a 20° angle under pentobarbital anesthesia (65 mg/kg, i.p.). Stereotaxic coordinates (in mm from bregma) were: AP=−7.8, LM=±3.1, DV=−7.0, respectively (Paxinos and Watson, 1998); cannulae were affixed to the skull with dental acrylic and jewelers' screws. Experimental procedures were done

Table 1. Effect of intra-MRN injection of saline vehicle (0.5 μl), 8-OH-DPAT (1 μg), muscimol (25 ng) on c-*fos* mRNA in rat brain and on locomotor activity[a]

Site	A-P (mm)	Area (mm^2)	Vehicle	8-OH-DPAT	Muscimol	*df*	*F*	*P*
MFC	+2.7	2.18	66643±4782	119833±13864†	99455±8672†	2,16	7.22	.006*
ACg	+1.7	1.37	42129±4317	66223±9349	59321±8043	2,16	2.79	.091
NAc	+2.2	1.37	11962±2060	26701±7621†	24197±3141†	2,16	3.85	.043*
CPu	+0.7	1.16	12855±2006	20547±5993	18737±3718	2,16	1.08	.362
MS	+0.7	0.77	6423±1082	11084±1761	7830±1474	2,16	2.47	.116
LS	+0.7	1.21	12329±2336	23740±3079†	19395±1981†	2,16	4.99	.021*
dBST	−0.3	0.94	4417±461	9178±1469†	8014±1284†	2,16	5.35	.017*
vBST	−0.3	0.67	3760±562	7003±1689	5207±1086	2,16	1.27	.307
PVN	−1.8	0.44	2322±464	4782±1211	3132±1043	2,16	1.68	.217
BLA	−2.6	0.44	7274±854	10715±1749	12301±1614†	2,16	3.71	.047*
CeA	−2.6	0.44	2683±607	4557±1400	3822±363	2,16	1.91	.179
dHipp	−3.6	0.44	8146±1360	11587±755	12718±2011	2,16	1.76	.203
vHIPP	−5.2	0.58	6093±575	11559±747†	9900±2400	2,15	4.86	.024*
VTA	−5.2	0.58	4382±1054	17016±5405†	15144±5688†	2,15	4.05	.039*
MRN	−7.3	0.44	9008±2234	12458±2126	10002±3747	2,16	.926	.417
LC	−9.7	0.29	1562±209	2555±492	3726±652†	2,16	4.52	.028*
Locomotor activity*			522±53	1002±103†	1190±270†	2,16	4.74	.024*

[a] The anterior–posterior location of each site is given in mm from bregma (Paxinos and Watson, 1998). The field sizes used to measure each site are given in mm^2. C-*fos* data are presented as group means±S.E.M. of the integrated optical density values determined for each brain region. Locomotor activity values reflect the group mean total photocell counts in the 30 min period following injection. Vehicle, $n=6–7$; 8-OH-DPAT, $n=6$; muscimol, $n=6$.

* Significant Drug effect, $P<0.05$.

† Significant differences from the Vehicle condition, $P<0.05$ (Fisher's LSD post hoc test).

in compliance with guidelines of the Canadian Council on Animal Care and those of the National Institutes of Health, USA (Publication 85-23, revised 1996). All efforts were made to minimize the number of animals used and their suffering.

Beginning 1 week after surgery, rats were handled daily on four occasions. Rats then received a 1 h habituation session in locomotor chambers (40 cm L×25 cm D×19 cm H) equipped with two photocells located 3 cm above the floor, spaced equally across the long axis of each box. The next day, animals were placed in the locomotor chambers for 30 min. They then received intra-MRN infusions of saline vehicle, 8-OH-DPAT or muscimol (Sigma, St. Louis, MO, USA; 1 μg and 25 ng, respectively, in 0.5 μl vehicle) through a 30-gauge injector; infusions were given over 45 s and the injectors were left in place for a further 30 s to allow diffusion. These doses were chosen as we have shown them to be effective in inducing the reinstatement of alcohol seeking and to significantly increase locomotor activity (Lê et al., 2002; Shim et al., 1997). Rats were replaced in the locomotor activity chambers for 30 min during which time photocell counts were recorded. Immediately afterward, rats were decapitated and their brains rapidly removed, frozen in isopentane (−40 °C), and stored at −70 °C. Brains were sliced coronally (12 μm) and were thaw-mounted on to glass slides coated with poly-L-lysine (Sigma). Cannula placements were verified histologically and five rats were excluded from analysis due to inaccurate placements.

In situ hybridization for c-*fos* mRNA was done as previously described (Funk et al., 2003). Briefly, the cRNA probe complementary to c-*fos* (680 mer; courtesy of Dr. T. Curran, St. Jude Children's Research Hospital, Memphis, TN, USA) was generated and labeled with [^{35}S]CTP and [35S]UTP (Amersham, Montreal, Canada). After overnight hybridization with the probe at 55 °C, brain sections were treated with RNAase A (200 μg/ml) for 1 h at 37 °C and washed to a final stringency of 0.1×SSC at 65 °C. After dehydration, slides were exposed to X-ray film (Kodak Biomax MR) for 6–10 days before developing.

Image capture and analysis was done as described previously (Funk et al., 2003). Results are expressed as integrated optical density values (mean gray value of pixels over threshold×number of pixels over threshold). Guided by a brain atlas (Paxinos and Watson, 1998), four to eight brain sections for each region were sampled bilaterally for each rat, and a mean integrated optical density value was determined for each site using a sampling area of standard size by a technician blind to the animals' treatment groups. For each brain region, the integrated optical density values were log-transformed and analyzed with separate ANOVAs, using the between-subjects factor of Drug (Vehicle, 1 μg 8-OH-DPAT, 25 ng muscimol). Significant effects were further analyzed by Fisher's least significant difference post hoc tests; the criterion for significance was $P<0.05$.

RESULTS

The effects of intra-MRN infusions of 8-OH-DPAT or muscimol on regional c-*fos* expression and locomotor activity are shown in Table 1. Compared with vehicle-injected animals, animals that received injections of 8-OH-DPAT or muscimol into the MRN showed significantly increased locomotor activity in the 30 min period after injection (Table 1, bottom). Both drugs induced comparable levels of locomotor activity. Injection of 8-OH-DPAT or muscimol into the MRN significantly increased the expression of c-*fos* mRNA in a number of brain areas (Table 1). The patterns of activation depended on the drug given and were regionally specific. ANOVA revealed a significant effect of drug administration in the MFC, NAc, lateral septum (LS), dBST, basolateral amygdala (BLA), vHIPP, VTA and locus coeruleus (LC). Post hoc analysis of these regions revealed that injections of either 8-OH-DPAT or muscimol significantly increased c-*fos* in the MFC, NAc, LS, dBST and VTA compared with vehicle. 8-OH-DPAT but not muscimol significantly increased c-*fos* in the vHIPP, while only muscimol injections significantly increased c-*fos* in the BLA and