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Review

Involvement of cholinergic neuronal systems in intravenous cocaine self-administration

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Abstract

Recent studies suggest the participation of cholinergic neurons in the brain processes underlying reinforcement. The involvement of cholinergic neurons in cocaine self-administration has been recently demonstrated in studies using muscarinic and nicotinic agonists and antagonists, microdialysis, assessment of choline acetyltransferase activity and acetylcholine (ACh) turnover rates. The present experiment was initiated to identify subsets of cholinergic neurons involved in the brain processes that underlie cocaine self-administration by lesioning discrete populations with a selective neurotoxin. Rats were trained to self-administer cocaine and the cholinergic neurotoxin 192-IgG-saporin or vehicle was then bilaterally administered into the posterior nucleus accumbens (NAcc)-ventral pallidum (VP). The 192-IgG-saporin induced lesions resulted in a pattern of drug-intake consistent with either a shift in the dose intake relationship to the left or downward compared to sham-treated controls. A second experiment used a self-administration threshold procedure that demonstrated this lesion shifted the dose intake relationship to the left compared to the sham-vehicle treated rats. The magnitude and extent of the lesion was assessed by measuring the expression of p75 (the target for 192-IgG-saporin) and choline acetyltransferase (ChAT) in the NAcc, VP, caudate nucleus-putamen (CP) and vertical limb of the medial septal nucleus-diagonal band (MS-DB) of these rats using real time reverse transcriptase-polymerase chain reaction. Significant reductions in gene expression for p75 (a selective marker for basal forebrain cholinergic neurons) and ChAT were seen in the MS-DB and VP while only small decreases were seen in the NAcc and CP of the 192-IgG-saporin treated rats. These data indicate that the overall influence of cholinergic neurons in the MS-DB and VP are inhibitory to the processes underlying cocaine self-administration and suggest that agonists directed toward subclasses of cholinergic receptors may have efficacy as pharmacotherapeutic adjuncts for the treatment of cocaine abuse.
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Keywords: Acetylcholine; Choline acetyltransferase; Drug reinforcement; 192-IgG-saporin and p75

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Abbreviations: ACh, acetylcholine; ChAT, choline acetyltransferase; CPP, conditioned place preference; CP, caudate nucleus putamen; D1, dopamine receptor subtype 1; DA, dopamine; MS-DB, vertical limb of the medial septum-diagonal band of Broca; Hipp, hippocampus; GABA, γ-aminobutyric acid; LH, lateral hypothalamus; M_1, muscarinic cholinergic receptor subtype 1; M_2, muscarinic cholinergic receptor subtype 2; M_4, muscarinic cholinergic receptor subtype 4; M_5, muscarinic cholinergic receptor subtype 5; NAcc, nucleus accumbens; VP, ventral pallidum; VTA, ventral tegmental area.

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1. Introduction

Cholinergic neurons in the basal forebrain are thought to have a role in the brain processes that underlie anxiety, arousal, attention, fatigue, sleep and a number of cognitive processes [3,12,16,55]. Selective lesions of cholinergic neurons in the diagonal band-preoptic region disrupt rat open field behaviors while such lesions of the septal region not only produce deficits in open field behaviors but also in nocturnal activity and delayed matching to sample performance indicating memory deficits [51]. In contrast, similar lesions of the nucleus basalis magnocellularis and medial septum cholinergic neurons do not alter spatial learning and memory in either the radial arm or water maze tasks [40] suggesting that subclasses of acetylcholine (ACh) releasing neurons may have different roles in brain function. In addition, cholinergic receptors have been shown to participate in the processes underlying brain stimulation reinforcement [61], and since drugs of abuse are thought to produce their addictive effects by activating such systems, cholinergic neurons may be involved in the processes underlying drug self-administration as well.

Although initial studies of the involvement of cholinergic neurons in cocaine self-administration found that receptor antagonists given systemically attenuated drug-taking in rats [19] and monkeys [20], these effects were thought to result from a non-specific behavioral disruption [13]. Recently, systemic pretreatment with a muscarinic cholinergic receptor mixed agonist-antagonist selectively decreased the reinforcing efficacy of intravenous cocaine in mice [44] while pretreatment with a nicotinic receptor antagonist produced similar effects in rats at doses that did not alter food-maintained responding [31]. In addition, ACh turnover rates were found to be selectively modulated in discrete brain regions of cocaine self-administering rats [49]. This experiment used a selective neurotoxin to destroy ACh-releasing neurons to assessed the role of discrete cholinergic innervations of/or interneurons in the nucleus accumbens (NAcc), ventral pallidum (VP), vertical limb of the medial septal nucleus-diagonal band (MS-DB) and caudate nucleus-putamen (CP) in intravenous cocaine self-administration.

2. Experimental procedures

2.1. Animals

Adult male Fischer F-344 rats (Harlan, Indianapolis, IN) 90–150 days old were used. The rats were housed in a temperature controlled environment on a reversed 12 h light–dark cycle (lights on 17:00–05:00). Food and water were available ad libitum except during experimental sessions. Experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) revised in 1996.

2.2. Surgical techniques

Intravenous catheters. Rats were anesthetized with pentobarbital (50 mg/kg, i.p.—Abbott Laboratories, North Chicago, IL) after pretreatment with atropine methyl nitrate (10 mg/kg, i.p.—Sigma, St Louis, MO) and Penicillin G procaine (75,000 units, i.m.—Wyeth Laboratories, Philadelphia, PA) and implanted with venous catheters placed in the right jugular vein using previously described methods [43,53,54]. The catheter (a small piece of polyvinyl-chloride tubing) was inserted into the right posterior facial vein, guided into the right jugular vein until it terminated just outside the right atrium and anchored to muscle in the area of the vein. The other end of the catheter continued subcutaneously to the back where it exited between the scapulae through a polyethylene shoulder harness. The harness provided a point of attachment for the catheter to a needle-tubing leash that passed out the top of the animal chamber. A leak proof swivel [7] was used to attach the leash to the tubing leading to the infusion pump so that the animals had almost complete freedom of movement. The rats were allowed to recover for 7–10 days before the initiation of experimental procedures. Automatically programmed infusions (0.2 ml delivered over 6.2 s) of heparinized saline (1.7 units/ml) were administered at hourly intervals in the home cage to maintain functional catheters. Patency of catheters was evaluated at regular intervals by delivering an intravenous infusion of methohexital (10 mg/kg-Eli Lilly, Indianapolis, IN) and determining the latency for loss of stability or consciousness which occurs within 1–2 s.

Intracranial injection guide cannula for microinjections. Intracranial guide cannulas were implanted bilaterally into the posterior NAcc–VP region of anesthetized animals (40 mg/kg pentobarbital, i.p.) using modifications of a previously described procedure [37]. Briefly, the procedure involved drilling appropriately placed holes (0.6 mm) in the calvarium and exposing the dura mater. Guide cannulas (26 gauge stainless steel tubing) were implanted stereotaxically to terminate just above the desired brain site using appropriate coordinates 7.4 mm rostral from lambda, 1.5 mm lateral from midline, 5.8 mm ventral from the brain [28]. The guide cannulas were permanently attached to the skull with stainless steel screws that were embedded in dental cement and stainless steel 32 gauge stylets were inserted.

2.3. Behavioral procedures

Cocaine self-administration. Rats ($N = 6$) with chronic jugular catheters were trained to intravenously self-administer cocaine (three doses of cocaine—0.08, 0.17 and 0.33 mg/infusion with the dose altered by changing

the pump activation duration) during daily $3\frac{1}{2}$ h sessions with 60 min exposure to each dose in a descending order and a 10 min time out (TO) between each component in modified operant chambers containing a house light, a stimulus light, response lever, and a tone source. A priming infusion of the dose to be available was delivered 20 s prior to each of the three components. The operant chambers were housed in sound and light-attenuating cubicles and daily self-administration sessions were conducted 5 days per week. Cocaine infusions were paired with a 20 s tone and light stimulus and the response lever was retracted during the TO periods and at the end of the $3\frac{1}{2}$ h sessions. The ratio requirement was raised to FR 2 after 5 days during which the mean number of infusions delivered for each dose of cocaine did not vary by more than 10% of the mean.

Thresholds for cocaine self-administration. Rats ($N = 8$) were trained to intravenously self-administer three doses of cocaine (0.17, 0.33 and 0.67 mg/infusion) on an FR 2 schedule as described above. When stable patterns of self-administration were obtained, the dose of cocaine was decreased by 50% sequentially over the next 10 sessions until a range of cocaine doses was found for which the rats would only self-administer the highest of the three available doses (0.02, 0.04 and 0.08 mg/infusion).

Food maintained responding. Rats were trained to lever press on an FR 2 schedule of food reinforcement during daily 1 h sessions in operant chambers identical to the self-administration chambers except that food pellet dispensers were present. The retractable lever was extended into the chamber and two responses resulted in delivery of a standard 45 mg food pellet (Research Diets, New Brunswick, NJ), which was immediately followed by the retraction of the lever and the presentation of a 20 s tone and light stimulus. The lever was extended immediately following the 20 s stimulus complex.

2.4. Cholinergic lesions procedures

Chemical lesions were produced by the microinjection of 192-IgG-saporin [40,48] through previously implanted cannulas. On the day of microinjection, the animals were anesthetized with methohexital, the stylets removed and injection cannula (32 gauge stainless steel tubing that extended 1.0 mm beyond the end of the guide cannula) inserted into the guide cannula which were in turn attached with PE tubing to 10 μl micro syringes. Rats received 1.0 μl of 0.25 μg of 192-IgG-saporin or vehicle into the posterior NAcc–VP region using a precision micro infusion pump at a rate of 0.3 μl/min. The injection cannula was removed 10 min after the drug delivery and the stylet replaced. The neurotoxin was dissolved in saline immediately before injection and saline alone was microinjected into sham-treated control animals.

2.5. Real-time RT-PCR

2.5.1. Tissue and RNA preparation

Rats were decapitated, brains removed and immediately frozen in isopentane (−30 to −40 °C). The brains were stored at −80 °C until microdissected into different areas at −20 °C. Total RNA was isolated using Tri-Reagent (Molecular Research Center). Briefly, homogenized tissue was placed in 1 ml Tri-reagent per 50–100 mg tissue and then incubated at room temperature. Chloroform (0.2 ml) was added and the reaction mixture incubated and centrifuged at 12,000*g* at 4 °C. RNA was precipitated by adding 0.5 ml isopropanol to the aqueous phase and centrifuged at 12,000*g*. The RNA pellet was rinsed once with 75% ethanol, air dried, resuspended in diethyl pyrocarbonate H_2O and incubated at 58 °C for 10 min. RNA concentration and quality were determined spectrophotometrically (ratio A260 nm/A280 nm = 1.8–2.0) and confirmed with gel electrophoresis.

Reverse transcription. One microgram of total RNA was reverse-transcribed using oligo dT primer and Omniscript RT kit [Qiagen, Valencia, CA]. The reaction mixture consisted of RT buffer, dNTPs (5 mM each), oligo dT primer, Omniscript reverse transcriptase and 1 μg of total RNA. The reaction was allowed to incubate at 37 °C for 1 h after which the Omniscript reverse transcriptase was inactivated by incubating at 95 °C for 5 min.

Real-time PCR. Gene specific primers and 5′-6 FAM, 3′-TAMRA labeled probes were designed using Primer Express Software 2.0 (Applied Biosystems, ABI) (Table 1). Gene sequences were accessed from the gene bank of the National Center for Biotechnology Information or from the literature, primers and probes purchased from ABI. TaqMan real-time RT-PCR assays for specific genes were analyzed using an ABI Prism 7000 Sequence Detection System (ABI). Each real-time PCR reaction consisted of TaqMan universal PCR master mix (ABI), gene specific forward and reverse primer, TaqMan probe and RT

Table 1
Primer and probe sequences for real-time RT-PCR

	ChAT	p75
Probe	CTCGGCAGCACTTCCAAGACACCAAT	CCCGGCTCCTTGGCCTGTTCTG
Forward primer	CCAGCCCTGCTGTGATCTTT	CGGTTCAGTGGCGCTTTC
Reverse primer	TGTAGCTAAGCACACCAGAGATGAG	CACTCATTCCAACAGCAAGCA

product from the previous step. The reaction started at 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, and annealing/extend at 60 °C for 1 min/cycle.

Data analysis. A four-fold dilution series of cDNA (1:0, 1:1, 1:3, 1:7) from each of the reverse transcription reactions was amplified. Relative quantification was determined from crossing threshold (C_t) values using a modification of the C_t method (ABI). Amplification efficiency was determined from the linear regression of the log cDNA for the dilution series versus the average C_t, where amplification efficiency (E) is equal to $E = 10^{(-1/slope)}$ [41]. The amplification efficiency was considered to be equal when the difference of efficiency between two groups was less than 0.1. Relative mRNA levels were determined by raising the amplification efficiency to the power of the difference between the C_t of the treated and control samples.

2.6. Data analysis

To identify changes in food-reinforced responding following 192-IgG-saporin-induced lesions, pellets delivered per session were averaged over sequential five-session blocks for each animal. Each block corresponded to one week of data collection. These means were entered into a one-way repeated measures analysis of variance (ANOVA) with time factor (one pre-lesion and three post-lesion time points).

Effects of 192-IgG-saporin-induced lesions on cocaine self-administration were also analyzed based on means of weekly five-session blocks. Each block mean was entered into a $2 \times 5 \times 3$ ANOVA with lesion (saline vs. 192-IgG-saporin), repeated time (one pre-lesion and four post-lesion time points), and repeated cocaine dose (0.02 vs. 0.04 vs. 0.08 mg/inf) factors. Significant interactions were analyzed with post-hoc Dunn's tests using the Bonferroni correction.

One-sample t tests were used to identify significant changes in p75 and ChAT gene expression following 192-IgG-saporin-induced lesions that shifted cocaine self-administration dose intake thresholds. For each animal, triplicate assays of fluorescence post-192-IgG-saporin-lesion were averaged within the NAcc, MS–DB, CP, and VP, divided by post-vehicle fluorescence, and multiplied by 100. These values were entered into a one-sample t test with 100 as the hypothesized value of the mean.

3. Results

Effects of 192-IgG-Saporin lesions of the posterior NAcc–VP on cocaine self-administration. 192-IgG-saporin-induced lesions of the posterior NAcc–VP decreased drug intake at all three doses compared to the sham-vehicle treated controls (Fig. 1). This effect was clearly seen by the fourth day post-treatment and was still present after 24 days. The pattern of drug intake after the saporin lesion is consistent with either a shift in the dose-intake relationship to the left or downward compared to the sham vehicle treatment.

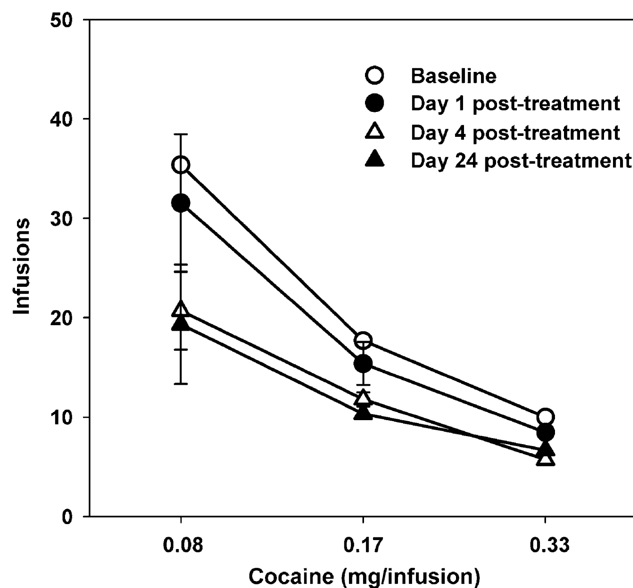

Fig. 1. Effects of 192-IgG-saporin lesions of the nucleus accumbens-ventral pallidum on the self-administration of cocaine. Rats daily self-administering three doses cocaine using a within session dose intake procedure received 192-IgG-saporin ($N = 6$) injections bilaterally into the posterior nucleus accumbens-ventral pallidum. The lesion shifted the dose intake relationship downward and this effect persisted for at least three weeks. Baseline data were obtained from the 5 sessions immediately preceding the lesion.

Effects of 192-IgG-saporin lesions of the posterior NAcc–VP on food-reinforced responding. 192-IgG-saporin-induced lesions of the posterior NAcc–VP did not significantly alter food-maintained responding (Fig. 2).

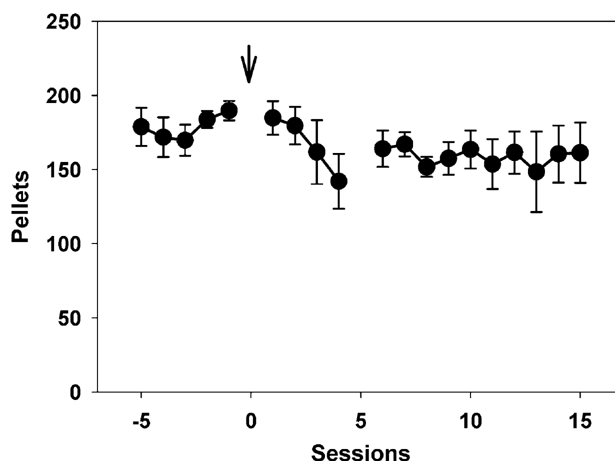

Fig. 2. Effects of 192-IgG-saporin lesions of the nucleus accumbens-ventral pallidum on food maintained responding. Rats were trained to lever press on an FR 2 schedule of food pellet presentation and received bilateral injections of 192-IgG-saporin ($N = 4$) into the posterior nucleus accumbens-ventral pallidum and the effects upon food intake monitored for 15 days. The lesion had only a modest transient effect on food maintained responding. The arrow represents when the lesion was made. Animals were not tested on the fifth day post-lesion because of conditions beyond the control of investigators.

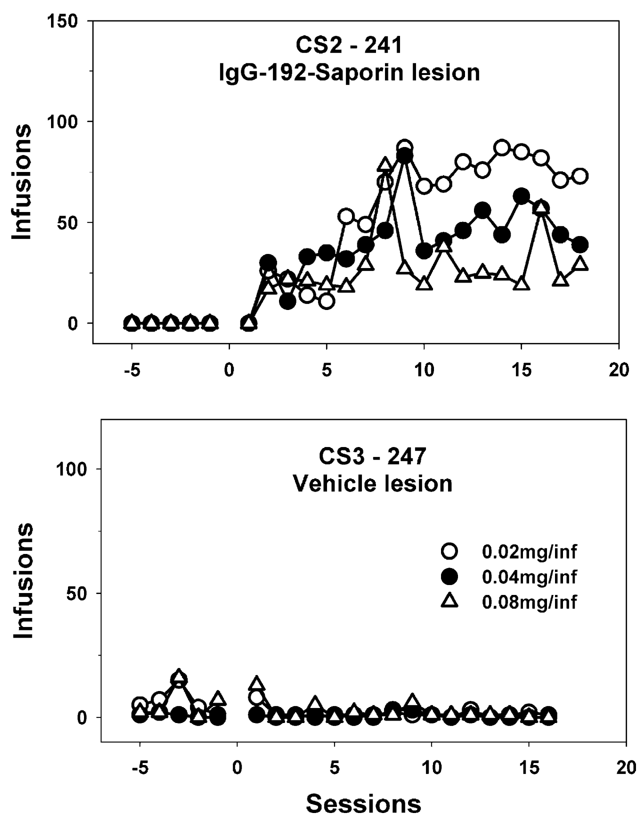

Fig. 3. Effects of 192-IgG-saporin lesions of the nucleus accumbens-ventral pallidum on cocaine self-administration thresholds. A pair of representative rats responding at threshold for cocaine self-administration were administered either 192-IgG-saporin (animal # CS3-247, top panel) or vehicle (animal # CS2-241, bottom panel) bilaterally into the posterior nucleus accumbens-ventral pallidum and the effects upon drug intake determined. The lesion shifted the dose intake relationship to the left with the lesioned animal self-administering doses below the pre-lesion threshold in a dose related manner.

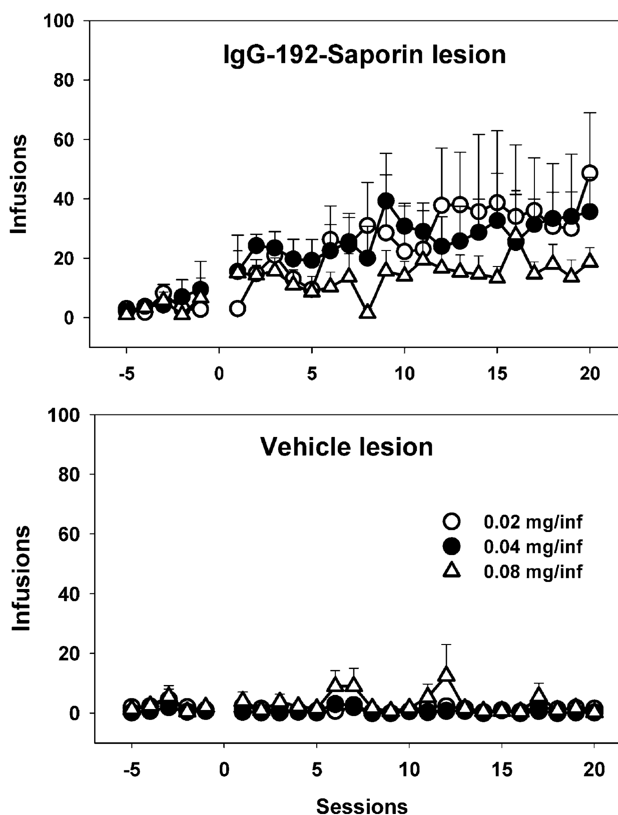

Fig. 4. Effects of 192-IgG-saporin or vehicle lesions of the nucleus accumbens-ventral pallidum on cocaine self-administration thresholds. Rats responding at the threshold for cocaine self-administration received either 192-IgG-saporin (top panel) or vehicle (bottom panel) lesions of the posterior nucleus accumbens-ventral pallidum. Sessions − 5 to − 1 were prelesion and the lesion was performed after the session on day 5. The points represent means and the error bars standard errors of the mean for an $n = 4$ in each condition. The lesion shifted the dose intake relationship for cocaine to the left resulting in stable self-administration of all three doses of cocaine while sham-vehicle treatment did not alter cocaine self-administration thresholds.

Effects of 192-IgG-Saporin lesions of the posterior NAcc–VP on cocaine self-administration. Six days after the 192-IgG-saporin-induced lesion of the posterior NAcc-VP, self-administration of doses of cocaine that would not maintain responding prior to the lesion began to occur (see top panel of Fig. 3 for data from a representative rat). This range of cocaine doses did not maintain responding in animals administered vehicle (see bottom panel of Fig. 3 for data from a representative rat).

A significant three-way interaction effect of lesion, time, and dose was found on cocaine self-administration, $F(8,40) = 2.2$, $p < 0.05$. The 192-IgG-saporin induced lesion of the posterior NAcc–VP resulted in the self-administration of 0.02 and 0.04 mg/inf beginning in the second post-lesion five-session block (starting on session 6, top panel, Fig. 4) and continuing through the fourth post-lesion five-session block (ending on session 20). There was no significant difference between the infusions of these two doses. Occasional increases in self-administration of the 0.08 mg/inf dose (Fig. 4) were not statistically significant following either lesion.

Effects of 192-IgG-Saporin lesions of the posterior NAcc–VP that shifted the cocaine self-administration dose intake thresholds on p75 and ChAT gene expression. The lesion resulted in a significant corresponding decrease in the DB of both p75 ($t(2) = 16.5$, $p < 0.01$) and ChAT ($t(3) = 47.1$, $p < 0.001$). Significant corresponding decreases were also found in the VP of both p75 ($t(3) = 38.7$, $p < 0.0001$) and ChAT ($t(2) = 10.1$, $p < 0.01$). There were slight decreases that approached statistical significance in the CP of p75 ($t(3) = 3.0$, $p = 0.06$) and ChAT ($t(3) = 2.5$, $p = 0.09$). Fig. 5 shows the real-time RT-PCR data for a pair of animals and Fig. 6 shows the normalized ratios for all four pairs.

4. Discussion

Neurotoxin induced lesions of the posterior NAcc–VP–MS-DB region shifted the cocaine self-administration dose intake relationship to the left compared to sham treated

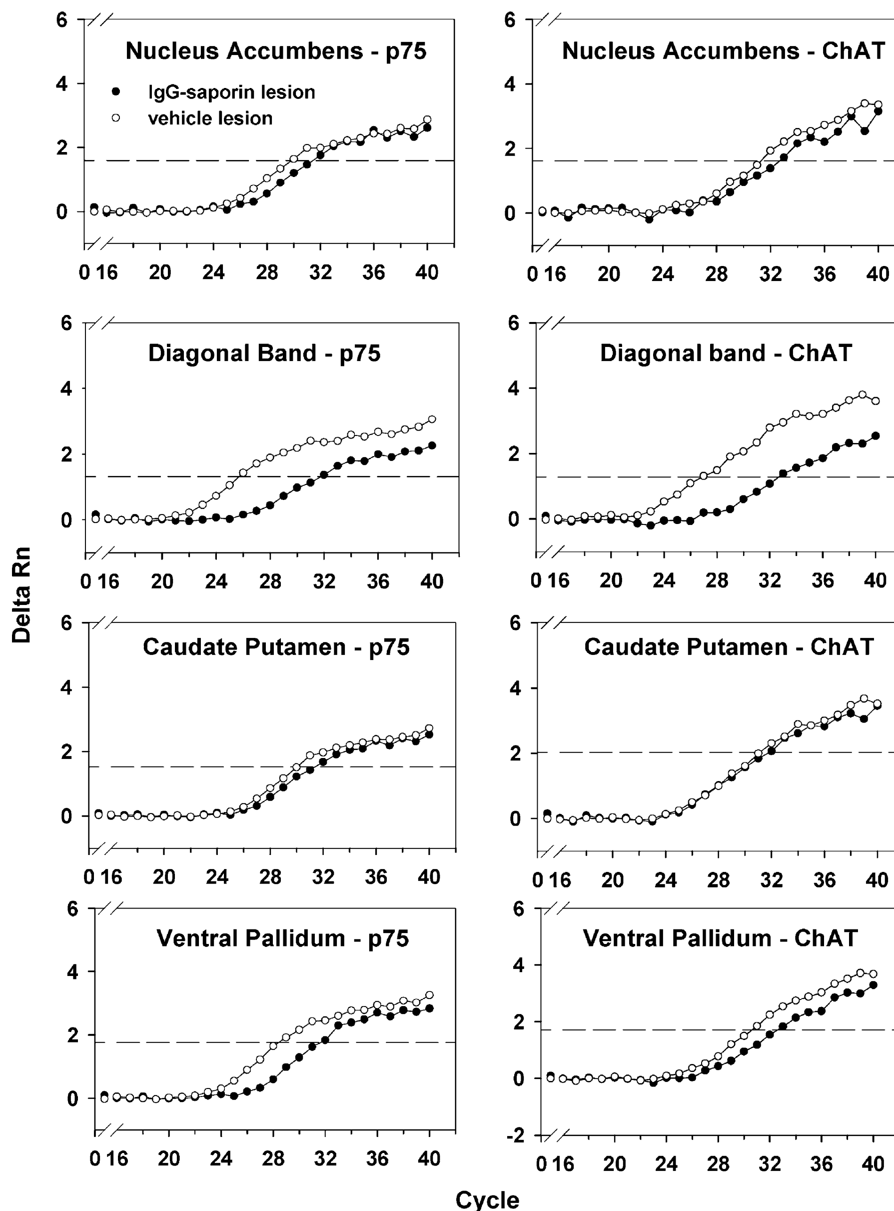

Fig. 5. Real-Time RT-PCR for p75 and choline acetyltransferase in the nucleus accumbens, diagonal band of Broca, caudate putamen and ventral pallidum. Data are from a pair of rats where one received 192-IgG-saporin (closed circles) and the other sham-vehicle treatment (open circles). The 192-IgG-saporin lesion decreased p75 and ChAT gene expression in the diagonal band of Broca and the ventral pallidum with very modest changes in the caudate-putamen and nucleus accumbens. Delta Rn represents the gene specific fluorescence signal minus the passive fluorescence signal (background).

controls. Real time RT-PCR showed significant reductions in p75 and ChAT gene expression in the MS-DB, VP and NAcc of the saporin treated rats. These data indicate that the net impact of cholinergic neurons in these three brain regions is inhibitory to the processes underlying cocaine self-administration. p75 has been assumed to be a selective marker for cholinergic neurons in the basal forebrain [59] and not associated with other cholinergic interneurons in the CP, NAcc and VP. These findings were based on data obtained with immunohistochemical techniques for p75 that had limited sensitivity for regions where cholinergic neurons are less dense. The real-time RT-PCR procedure used here was more sensitive, permitting identification of p75 in the CP, NAcc and VP (Figs. 5 and 6). However, whether this low affinity nerve growth factor receptor, p75 is associated exclusively on cholinergic neurons in these brain regions is yet to be demonstrated.

Although ChAT activity in the NAcc was not significantly reduced in the 192-IgG-saporin lesioned rats compared to the sham treated controls (Fig. 6), ChAT activity was previously been shown to be reduced in the NAcc of rats on the last day of cocaine self-administration and after 3 weeks of withdrawal [57] indicating a down regulation of cholinergic tone in this structure. In addition, the selective removal of cholinergic interneurons in the NAcc enhanced cocaine induced locomotion and

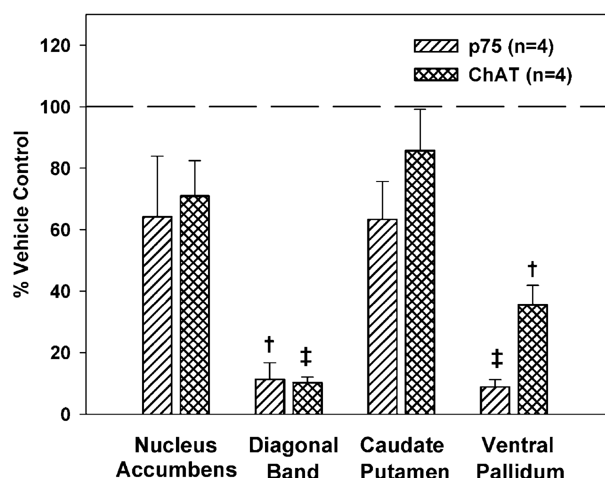

Fig. 6. p75 and choline acetyltransferase gene expression. p75 and choline acetyltransferase gene expression in four brain regions of four pairs of rats intravenously self-administering cocaine that received either 192-IgG-saporin of sham vehicle treatment into the posterior nucleus accumbens-ventral pallidum. The diagonal band of Broca and the ventral pallidum showed significant deceases in both. These are the same animals whose behavioral data are shown in Fig. 4. Significance of difference between means are: $\dagger = p < 0.01$; $\ddagger = p < 0.001$.

conditioned place preference (CPP) in mice [23] suggesting an inhibitory role for ACh in this structure in the cellular events underlying these behaviors. Increased extracellular fluid levels of ACh were seen in microdialysates of the NAcc during cocaine self-administration compared to yoked cocaine infused rats [33]. Collectively, these studies indicate an involvement of ACh interneurons in the NAcc in the brain processes that underlie cocaine self-administration.

Although cholinergic neurons comprise only 2% of the total neuronal population of the NAcc and striatum, these cells have a prominent role in modulating the activity of GABAergic medium spiny neurons, which comprise 90–95% of the neuronal population of these brain regions [65]. Cholinergic receptors in the NAcc have been shown to modulate the activity of inhibitory GABAergic transmission. Nicotinic cholinergic receptor agonists potentiate GABAergic transmission within the NAcc that partially determines the magnitude of the inhibition produced by the medium spiny output neurons [36]. Agonists for, these receptors enhance GABAergic inhibition of the medium spiny output neurons while muscarinic cholinergic receptor agonists suppresses this effect [14]. The effects upon cocaine self-administration observed here likely involve modulation of the medium spiny neurons through both muscarinic and nicotinic cholinergic receptor subtypes. Muscarinic receptor activation results in both excitatory and inhibitory post-synaptic potentials. M3 receptors inhibit and M_5 receptors stimulate DA release through receptors located on DA terminals, while M_4 receptors likely stimulate DA release indirectly through inhibition of GABAergic medium spiny neurons resulting in a reduced $GABA_A$ receptor inhibition of DA release [63]. Muscarinic blockade of cholinergic receptors in the NAcc did not affect established cocaine self-administration in rats [24]. However, this effect could result from antagonism of both excitatory (M_5) and inhibitory (M_3 and M_4) cholinergic processes in this structure [63].

Nicotinic receptors are also dense in these two brain regions and likely have a role in the effects of the192-IgG-saporin lesion on cocaine self-administration. β_2 nicotinic receptors appear to be involved in the processes underlying the reinforcing properties of cocaine since transgenic mice lacking the high affinity β_2 subunit showed decreased CPP to cocaine [62]. However, intravenous cocaine self-administration occurred at the same rate as the wild type in this knockout [15] while nicotine self-administration was attenuated [42]. Nicotinic agonists have been demonstrated to stimulate NAcc DA release assessed with microdialysis [34] and from purified synaptosomes prepared from the NAcc and striatum [47,58]. Evoked striatal DA release was suppressed in mutant mice lacking the β_2 nicotinic receptor subunit [64]. Decreases in DA release have been shown to result from activation of both $\alpha_4\beta_2$ [22] and the α_7 homomeric nicotinic receptor which is thought to be the most relevant entity for the direct effects of nicotinic modulation within the NAcc [17]. The α_7 receptor can have both excitatory and inhibitory effects in this structure that appear to be dependent upon the loci of the receptor [4]. The α_7 receptors in the ventral tegmental area appear to mediate the enhancing effects of cocaine upon brain stimulation reinforcement [39] further supporting a role in stimulant self-administration. However, the involvement of these receptor subtypes in the self-administration of cocaine is yet to be assessed directly.

The shift to the left in the dose-intake function for cocaine following the 192-IgG-saporin lesion further demonstrates the importance of DA-ACh interactions in brain function. There has been an increase in understanding of the role of cholinergic interneurons and their relationship to dopaminergic afferents in the forebrain [65]. Dopaminergic fibers from the VTA synapse directly on cholinergic interneurons as well as medium spiny neurons [8,29] with DA modulating the activity of these neurons through voltage-dependent ion channels as well as through excitatory and inhibitory synaptic actions [38]. Generally, D_1-like receptors increase and D_2-like receptors decrease membrane excitability in the striatum [56]. D_2 receptors are the predominant receptor subtype on striatal cholinergic interneurons with D_5 receptors also present [65]. D_3 receptors may be located on cholinergic interneurons in the NAcc as well since selective antagonists enhance evoked DA release in this structure [60]. Both excitatory (D_1 and D_5) and inhibitory (D_2) effects of DA on cholinergic interneurons have been observed. Decreased release of ACh from cholinergic interneurons has been shown by activation of D_2 and D_3 receptors [50,60] and increased release occurs through feed-forward inhibition of dopaminergic circuits by activation of D_1 and D_5 receptors [9,33].

The significant decreases in p75 and ChAT in the MS-DB in the saporin lesioned animals suggests that cholinergic neurons in this region may produce a negative feedback on the actions of cocaine through dopaminergic neurons. The MS-DB neurons receive excitatory glutamatergic input from the entorhinal cortex [1], inhibitory GABAergic input from the hippocampus [52] and both monoaminergic and peptidergic input from the brainstem [11,26,35]. There are also cholinergic interneurons within the MS-DB that synapse onto cholinergic projection neurons in this structure [5,30,35]. The output of the MS-DB consists almost exclusively of cholinergic and GABAergic projection neurons, with the cholinergic afferents comprising approximately two-thirds of the neuronal population [6,27]. These projection neurons innervate the hippocampus, olfactory bulb and the entire cortical mantle [25]. A septo-hippocampal-septal loop appears to serve as a feedback inhibition from the hippocampus to the MS-DB that contains GABAergic projections from the hippocampus that synapse onto cholinergic projection neurons within the MS-DB [52]. Increased turnover rates of ACh were seen in the hippocampus of cocaine self-administering rats [49] suggesting that the balance in this loop shifts toward the activation of cholinergic neurons that innervate this structure. The interaction of MS-DB neurons with the monoaminergic efferents from the brainstem are complex, with noradrenergic neurons producing excitation of cholinergic MS-DB neurons and dopaminergic inputs producing inhibition of these cells [10,32,45]. The substantia nigra and VTA send dopaminergic projections that synapse onto cholinergic neurons in the MS-DB [18] and the MS-DB contains relatively dense populations of D_3 receptors [2]. It appears that the effects of enhanced dopaminergic tone seen with cocaine self-administration are overshadowed by other systems since an increase in ACh turnover occurs in the hippocampus [49]. Electrolytic lesions of the septum and MS-DB regions increases the sensitivity of rats to cocaine in CPP, suggesting an inhibitory modulation of cocaine reinforcement by this region [21] that is consistent with the present data.

The data from these experiments indicate that cholinergic innervations of the VP, MS–DB and NAcc are inhibitory to the brain processes that underlie cocaine self-administration. In addition, the nucleus basalis magnocellularis which contains cell bodies for a major cholinergic system innervating the forebrain may also be involved since alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid induced lesions shifted the cocaine dose-intake relationship to the left suggesting an enhancement of reinforcing efficacy [46], which is consistent with the role of these neurons in the MS-DB, NAcc and VP. Identification of the specific cholinergic receptors that are responsible for this shift to the left in the dose intake function for cocaine self-administration could provide potential targets for pharmacotherapeutic adjuncts for the treatment of cocaine abuse. In addition, since drugs of abuse are thought to have addictive properties because they modulate brain systems dedicated to reinforcement and since cholinergic receptors have been shown to participate in the processes underlying brain stimulation reinforcement [39,61], it is likely that some of the cholinergic neurons identified here represent neuronal systems mediating reinforcement in general and thus may have broader applications to understanding the biological basis of behavior.

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