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Role of muscarinic receptors in the activation of the ventral subiculum and the consequences for dopamine release in the nucleus accumbens

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Abstract

The nucleus accumbens receives limbic inputs from a number of brain regions, including the ventral subiculum. In rats, activation of the ventral subiculum following microinjection of *N*-methyl-D-aspartate (NMDA) or carbachol increases locomotor activity, whilst ventral subiculum application of NMDA also increases dopamine efflux in the ipsilateral nucleus accumbens. Microdialysis experiments were therefore conducted to ascertain the consequences for dopamine release in the nucleus accumbens following ventral subiculum administration of carbachol, and to explore the acetylcholine receptor subtype(s) that might be involved. We report that, in anaesthetised rats, ventral subiculum administration of carbachol increased dopamine levels in the nucleus accumbens. The response was attenuated by co-administration with atropine, whilst administration of nicotine and the α-7 nicotinic acetylcholine receptor agonist AR-R17779 (spiro[1-azabicyclo[2,2,2]octane-3,5′-oxazolidine]-2′-one monohydrochloride) failed to evoke a response. Oxotremorine-M produced a dose-dependent increase in dopamine efflux confirming sensitivity to muscarinic receptor stimulation. However, the ventral subiculum was insensitive to xanomeline and pilocarpine, muscarinic M_1 receptor-preferring agonists, but sensitive to BuTAC ([5R-[exo]-6-[butylthio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1])octane), a muscarinic M_2/M_4 receptor agonist. The dopamine response to oxotremorine-M was significantly attenuated, although not abolished by co-administration with the M_2/M_4 receptor antagonist methoctramine, and studies combining oxotremorine-M with (−)-bicuculline, indicated a dual action in the ventral subiculum that was dependent and independent of reduced GABA neurotransmission. The data presented indicates that activation of the ventral subiculum by carbachol increases dopamine efflux in the nucleus accumbens by stimulation of muscarinic receptors, and that the ventral subiculum–nucleus accumbens projection system is sensitive to muscarinic M_2/M_4 receptor stimulation.

Keywords: Microdialysis; Ventral subiculum; Muscarinic receptor; Nucleus accumbens; Dopamine; (Rat)

1. Introduction

Neuroanatomical studies have revealed that the nucleus accumbens receives projections from limbic structures, including the hippocampus, amygdala and temporal cortex (Kelley and Domesick, 1982; Kelley et al., 1982; Groenewegen et al., 1987; Sesack and Pickel, 1990a), and that these projections appear to be glutamatergic (Christie et al., 1987; DeFrance et al., 1980; Fuller et al., 1987). One projection in particular, from the ventral hippocampus (which includes the ventral subiculum), innervates the nucleus accumbens shell sub-region (Groenewegen et al., 1987; Totterdell and Smith, 1989; Sesack and Pickel, 1990b). Activation of the ventral subiculum, following microinjection of *N*-methyl-D-aspartate (NMDA) or carbachol into the ventral hippocampus has been reported to increase locomotor activity (Yang and Mogenson, 1987; Wu and Brudzynski, 1995; Brenner and Bardgett, 1998). These studies supported neuroanatomical data implying a specific interaction between glutamatergic and dopaminergic systems, and suggested that the behavioural response was a consequence of elevated levels of dopamine. More recently, microdialysis studies have been used to monitor transmitter release directly. In these experiments, ventral subiculum administration of NMDA was found to increase extracellular levels of dopamine in the nucleus accumbens (Brudzynski and Gibson, 1997; Legault and Wise, 1999; Legault et al., 2000; Mitchell et al., 2000b).

The subiculum receives a major cholinergic innervation, and a direct muscarinic modulation of subicular neurones

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has been reported (Kawasaki et al., 1999). In these studies, application of the acetylcholine receptor agonist carbachol to subicular neurones in vitro evoked a profound increase in intrinsic excitability that was reversed by atropine (Kawasaki et al., 1999). It is anticipated that carbachol-induced changes in the excitability of subicular neurones in vivo may have consequences for the overall activity of the subiculum projection system, leading to increased dopamine release in the nucleus accumbens. Such a response would also provide neurochemical support for the finding of a dopamine-sensitive increase in locomotor activity following ventral subiculum application of carbachol (Brenner and Bardgett, 1998).

In this paper we describe experiments that were undertaken, using in vivo microdialysis, to explore further the neurochemical consequences, within the nucleus accumbens, of ventral subiculum administration of carbachol. Since carbachol is a non-selective acetylcholine receptor agonist, experiments were also undertaken to explore the potential contribution of nicotinic or muscarinic receptors, using selective agonists and antagonists, in the regulation of the ventral subiculum–nucleus accumbens projection system.

2. Materials and methods

2.1. Animals

Lister Hooded rats (male; 290–320 g, Harlan, UK) used in these experiments were housed on a 12/12 h dark/light cycle; food and water were available ad libitum. The procedures for animal care and use were in accordance with the Animals (Scientific Procedures) Act 1986.

2.2. Brain surgery and microdialysis

Male Lister Hooded rats (Harlan, 280–300 g) were anaesthetised with urethane (1.3 g/5 ml 0.9% saline; 5 ml/kg i.p.) and positioned in a stereotaxic frame; body temperature was maintained at 36–37 °C using a heated pad and temperature controller.

Holes were drilled into the skull to allow placement of an injection cannula into the left ventral subiculum, and a microdialysis probe (Hospal ANH69, active length 2 mm) into the ipsilateral nucleus accumbens shell (Louilot and Le Moal, 1994). Coordinates for surgical implantation were (from bregma and dura surface, and with bregma and lambda in the same vertical plane), nucleus accumbens: AP +1.5, LM −0.8; DV −7.0 mm; ventral subiculum: AP −6.3, LM +4.8; DV −5.5 mm (from the atlas of Paxinos and Watson, 1998).

Microdialysis probes were implanted whilst being perfused at a flow-rate of 1.5 μl/min with artificial cerebrospinal fluid (aCSF), composition (in mM): NaCl (141), KCl (5), $MgCl_2$ (0.8), $CaCl_2$ (1.5), Na_2HPO_4/NaH_2PO_4 (1.4/0.25) and 10 μM ascorbic acid (pH 7.4). Ninety minutes after probe implantation, samples were collected every 15 min in vials containing 5 μl preservative (0.33 mM L-cysteine, 0.22 mM ethylenediaminetetra acetic acid, 50 μM ascorbic acid and 0.1 M glacial acetic acid). After a further 90 min, an injection cannula (33 gauge), loaded with drug or vehicle, was implanted into the ventral subiculum. Drug or vehicle (aCSF) was administered in 1 μl over 2 min, 30 min later, and samples collected for a further 2–3 h.

At the end of the experiment, the injection cannula was removed, Fast Green (1%) loaded and re-injected (0.15 μl). The animal was then decapitated and the brain removed and stored in 10% formal-saline for subsequent histological verification of both microdialysis probe and injection placements. Data was used from animals in which both microdialysis probe and injection cannula placements were verified to be in the appropriate brain regions. Fig. 1A and B shows results of histological evaluation of microdialysis probe and injection cannula placements in the nucleus accumbens shell and ventral subiculum, respectively.

2.3. High-performance liquid chromatography details

The high performance liquid chromatography (HPLC) system consisted of a Rheos 4000 pump (Presearch), an on-line degasser, a Hypersil BDS analytical column (150 × 3 mm, 5 μm C_{18}, Thermoquest), and a Triathlon autosampler (Presearch). Detection of dopamine and its metabolites was accomplished with an Antec electrochemical detector (Presearch) with a glass-carbon electrode maintained at +0.750 V versus an Ag/AgCl reference electrode. Chromatographic separation and electrochemical detection were performed at 35 °C. The mobile phase consisted of a 75 mM phosphate buffer, containing 350 mg octane sulphonic acid, 7% acetonitrile, 0.7% tetrahydrofuran and 0.1 mM ethylenediaminetetra-acetic acid (pH 3.20). The flow rate was 400 μl/min. Chromatograms were displayed, integrated and stored using Millennium^{32} (Waters).

2.4. Data analysis

Data from experiments were converted from peak areas using a calibration curve and reported as nM. The three samples prior to drug or vehicle administration were averaged to yield a pre-injection control value (equivalent to 100%). All samples were expressed as a percentage of this control value. Differences in response to drug administration were analysed by Multivariate Analysis of Variance (with time as a repeated measure) following log-transformation of percentage data. Significance was taken at the 5% level (JMP version 4.0.2, SAS Institute, USA).

2.5. Materials

Atropine sulphate, (−)-bicuculline methobromide H_2O, carbamylcholine chloride (carbachol), methoctramine,

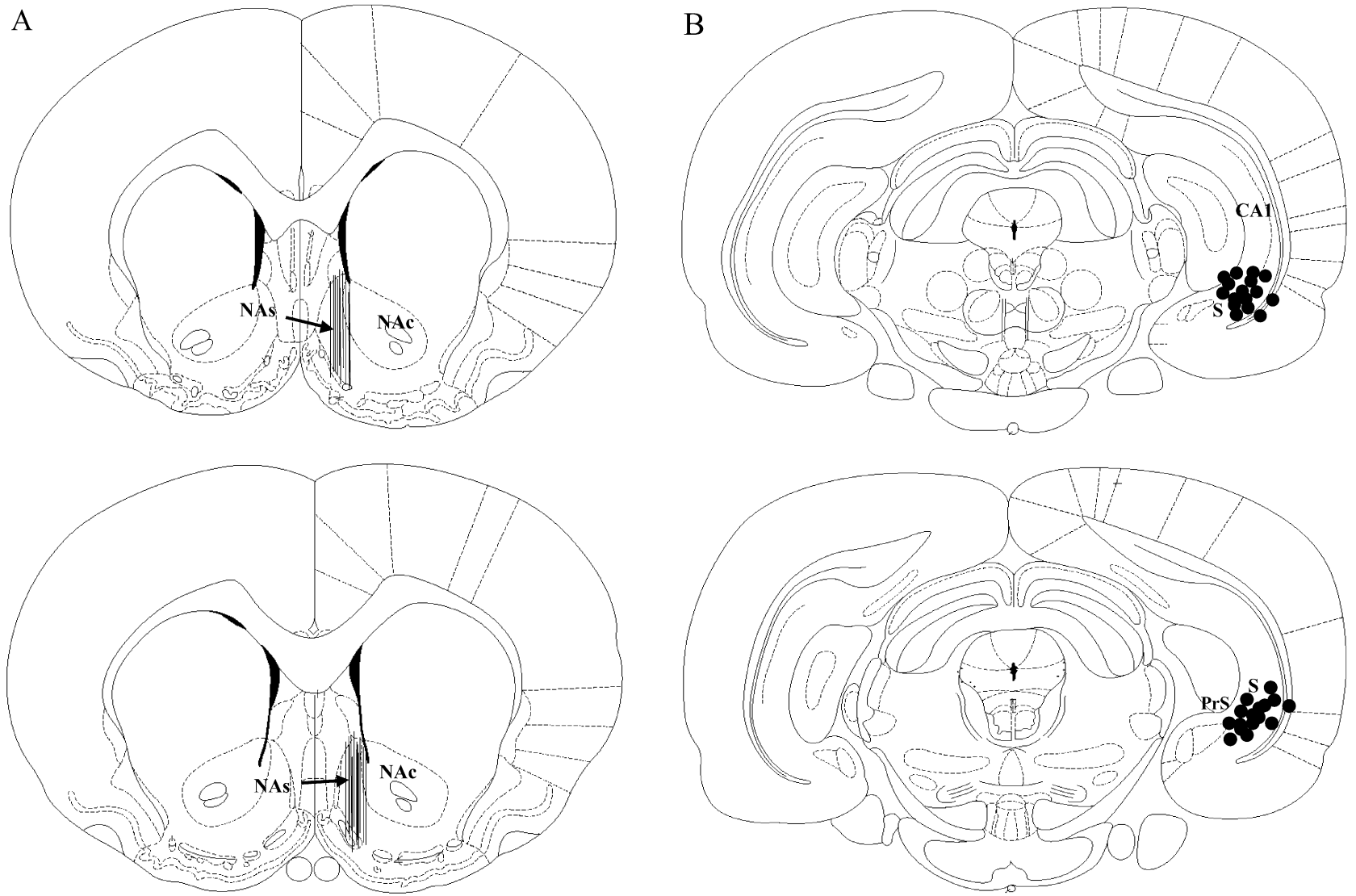

Fig. 1. Histological verification of microdialysis probe placements (A) in the nucleus accumbens shell (NAs) and injection sites (B) in the ventral subiculum (S). Plates were taken from Paxinos and Watson (1998) (NAc = nucleus accumbens core; PrS = presubiculum; CA1 = field CA1 of Ammon's horn).

(−)-nicotine bi-tartrate and pilocarpine were obtained from Sigma (Poole, UK), and oxotremorine-M from Tocris. AR-R17779 (spiro[1-azabicyclo[2,2,2]octane-3,5′-oxazolidine]-2′-one monohydrochloride), BuTAC (([5R-[exo]-6-[butylthio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1])octane) and xanomeline tartrate (3(3-hexyloxy-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine) were synthesised at Lilly Research Centre. All drugs were dissolved in aCSF (BuTAC was dissolved in a drop of lactic acid then resuspended in aCSF and adjusted to pH 5–6 with sodium hydroxide).

3. Results

3.1. Effect of ventral subiculum administration of carbachol, nicotine and AR-R17779 on dopamine levels in the nucleus accumbens

Administration of carbachol (500 ng in 1 μl) elicited a rapid increase in extracellular levels of dopamine in the ipsilateral nucleus accumbens, reaching 234 ± 53% of the pre-injection control within 30 min (Fig. 2; $F(1,9)=10.32$, $P=0.0106$). The response was maximal at this time, and levels declined to baseline after a further 30 min. Administration of either nicotine or the α-7 nicotinic acetylcholine receptor agonist AR-R17779 (Mullen et al., 2000), both at 500 ng in 1 μl, failed to evoke a dopamine response in the nucleus accumbens (Fig. 2).

3.2. Effect of atropine on the dopamine response to carbachol

The contribution of muscarinic receptors in the response to carbachol was examined by determining whether the evoked response could be prevented by administration of the muscarinic antagonist, atropine. In these experiments,

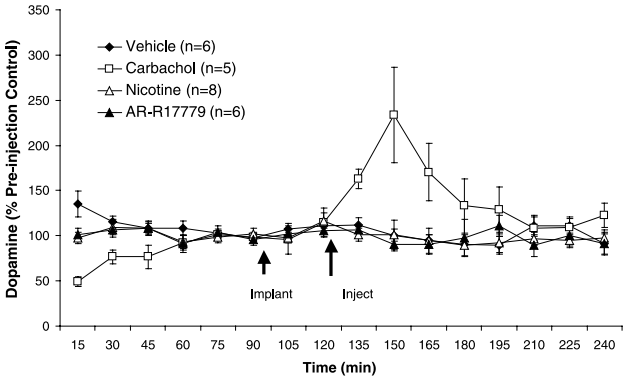

Fig. 2. Effect of ventral subiculum administration of acetylcholine receptor agonists on extracellular dopamine in the nucleus accumbens. Data expressed as a percentage of a pre-injection control (mean ± S.E.M.). Injection needle implanted into ventral subiculum at first arrow; drugs delivered (each at 500 ng) 30 min later at second arrow.

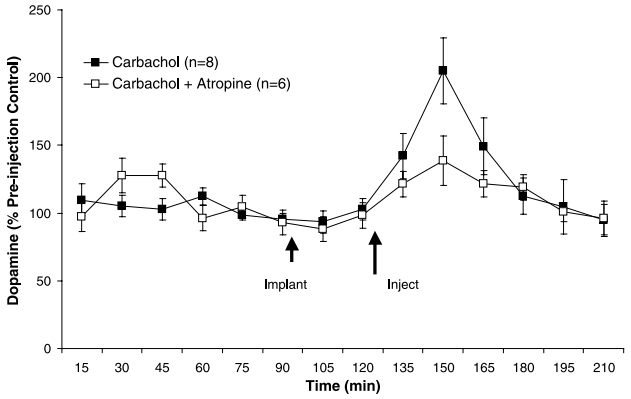

Fig. 3. Effect of atropine on the dopamine response elicited by ventral subiculum stimulation with carbachol. Data expressed as a percentage of a pre-injection control (mean ± S.E.M.). Injection needle implanted into ventral subiculum at first arrow; drugs delivered 30 min later at second arrow (either 500 ng carbachol, or co-application of carbachol with atropine, both at a dose of 500 ng).

co-administration of carbachol with atropine (both at 500 ng) significantly attenuated the maximal response obtained with carbachol alone (500 ng in 1 μl; Fig. 3; $F(1,12)=6.02$, $P=0.030$).

3.3. Effect of ventral subiculum administration of oxotremorine-M, pilocarpine, xanomeline and BuTAC on dopamine levels in the nucleus accumbens

Further exploration of the role of muscarinic receptors was undertaken using selective muscarinic agonists. Administration of the broad spectrum muscarinic receptor agonist oxotremorine-M, dose-dependently and significantly increased extracellular levels of dopamine in the nucleus accumbens (Fig. 4A; effect of Treatment: $F(2,13)=36.33$, $P<0.0001$). Levels of dopamine reached 325 ± 27% within 30 min after the highest dose (500 ng versus vehicle: $F(1,13)=72.33$, $P<0.0001$), and remained elevated for the duration of recording (for up to 2 h). After administration of the lower dose (100 ng in 1 μl), levels reached 206 ± 20% and returned to baseline 90–105 min later (100 ng versus vehicle: $F(1,13)=10.81$, $P=0.0059$).

Studies were then undertaken to explore the sensitivity of the subiculo–accumbens projection system to agonists with preferential selectivity for muscarinic receptor subtypes. These experiments were conducted with agonists showing preferential selectivity for the muscarinic M_1 receptor subtype, namely pilocarpine (muscarinic M_1 and M_3 preferring; Lisa Broad, personal communication) and xanomeline (Sauerberg et al., 1992; Bymaster et al., 1994, 1997; Shannon et al., 1994), and BuTAC, a partial agonist at muscarinic M_2 and M_4 receptors and an antagonist at muscarinic M_1, M_3 and M_5 receptors (Sauerberg et al., 1998; Shannon et al., 1999).

Administration of pilocarpine or xanomeline into the ventral subiculum (at 500 ng , 1 μg, or 5 μg in 1 μl) failed

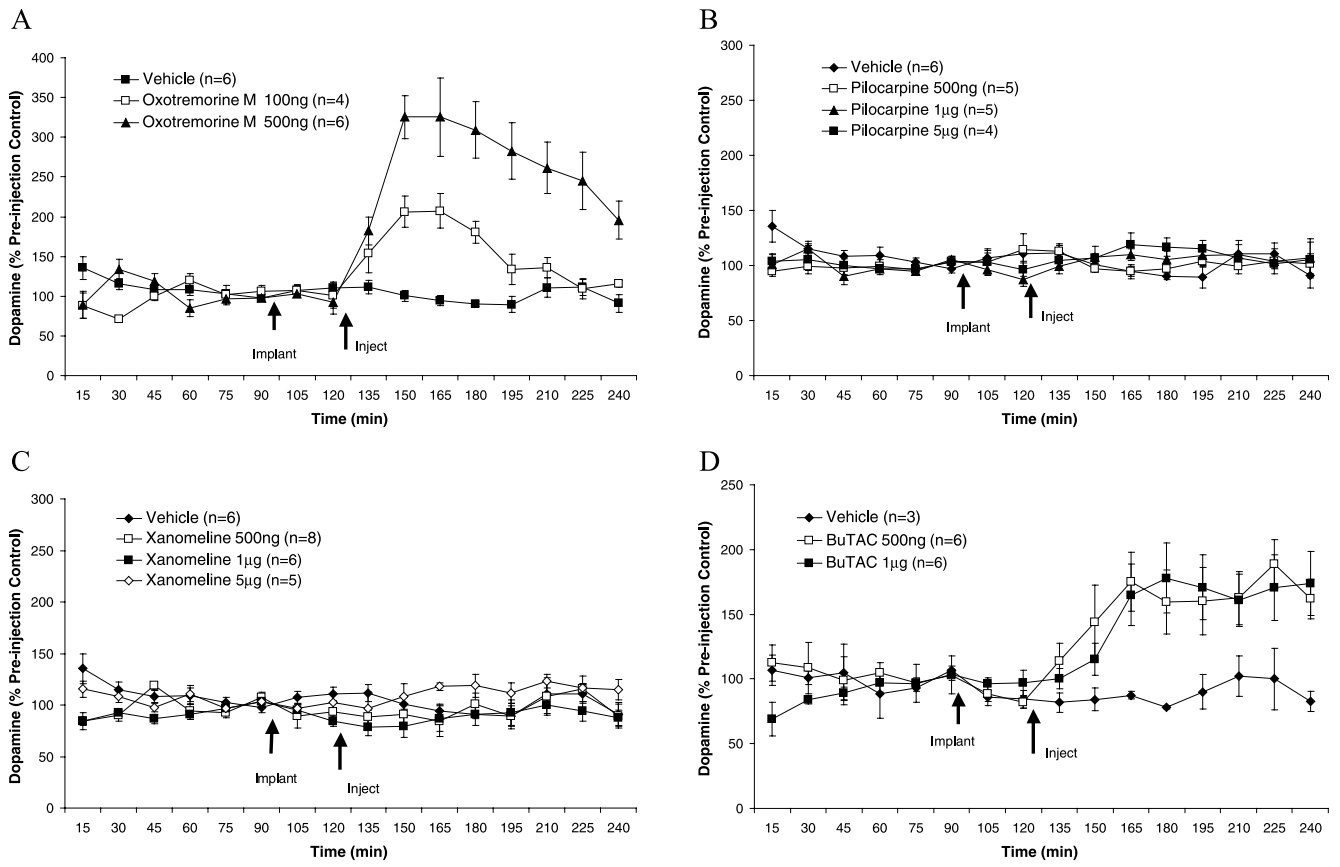

Fig. 4. Effect of ventral subiculum administration of (A) oxotremorine-M, (B) pilocarpine, (C) xanomeline and (D) BuTAC on extracellular dopamine in the nucleus accumbens. Data expressed as a percentage of a pre-injection control (mean ± S.E.M.). Injection needle implanted into ventral subiculum at first arrow; drugs delivered 30 min later at second arrow.

to significantly alter extracellular levels of dopamine in the nucleus accumbens (pilocarpine: $F(3,16)=0.40$, $P=0.75$; xanomeline: $F(3,21)=1.51$, $P=0.24$ Fig. 4B and C, respectively). However, intra-subicular administration of BuTAC (500 ng and 1 µg) evoked a significant response ($F(2,10)=5.54$, $P=0.024$; Fig. 4D). At a dose of 500 ng, extracellular levels of dopamine in the nucleus accumbens increased to approximately 167% of the pre-injection control (versus vehicle: $F(1,10)=9.86$, $P=0.0105$). The response appeared to be maximal as administration of a higher dose (1 µg) failed to evoke a greater response. At 1 µg, levels were increased to approximately 169% (versus vehicle: $F(1,10)=7.87$, $P=0.018$).

3.4. Effect of methoctramine on the dopamine response to ventral subiculum administration of oxotremorine-M

Examination of the role of muscarinic M_2 and/or M_4 receptor subtypes in the response to oxotremorine-M was undertaken using the muscarinic M_2/M_4 receptor antagonist methoctramine (Dörje et al., 1991). In combination with methoctramine at 10 or 50 µg, the response to oxotremorine-M (500 ng) was significantly reduced [$F(2,14)=5.08$, $P=0.0219$; Fig. 5]. Methoctramine significantly reduced the extent of the dopamine response from 259 ± 39% with oxtremorine-M alone to 159 ± 37% and 179 ± 21% (with respect to the pre-injection control) in combination with 10 and 50 µg, respectively, 45 min after administration (Fig. 5). (Control oxotremorine-M response versus oxotremorine-M

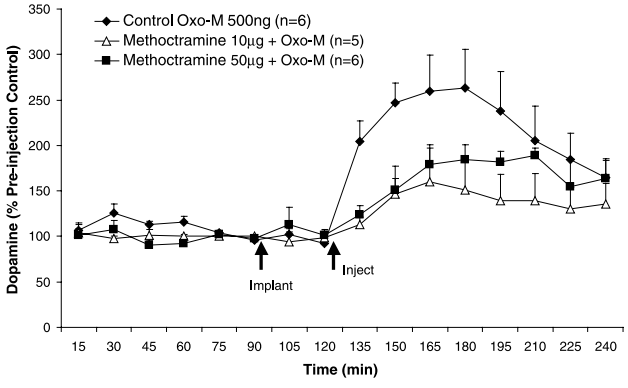

Fig. 5. Effect of the combined administration of methoctramine and oxotremorine-M on extracellular dopamine in the nucleus accumbens. Data expressed as a percentage of a pre-injection control (mean ± S.E.M.). Injection needle implanted into ventral subiculum at first arrow; drugs delivered 30 min later at second arrow.

plus10 μg methoctramine: $F(1,14)=9.32$, $P=0.0086$; plus 50 μg methoctramine: $F(1,14)=5.13$, $P=0.0399$). The reduction in the dopamine response to oxotremorine-M with methoctramine appeared to represent the maximal inhibition that could be attained by blocking muscarinic M_2/M_4 receptors.

3.5. Effect of ventral subiculum administration of (−)-bicuculline on dopamine levels in the nucleus accumbens, and on the response evoked by oxotremorine-M

Experiments were undertaken using the $GABA_A$ receptor antagonist (−)-bicuculline to firstly, explore the role GABA in the control of the ventral subiculum and secondly, in combination with oxotremorine-M, to elucidate whether a reduction in GABA neurotransmission may underlie the response to oxotremorine-M.

Administration of (−)-bicuculline at 5, 10 and 20 μg, dose-dependently increased extracellular levels of dopamine in the nucleus accumbens [$F(3,14)=18.37$, $P<0.0001$; Fig. 6]. The response evoked by (−)-bicuculline appeared to be maximal at 10 μg, with no further significant increase occurring with the 20-μg dose. The maximal response achieved with (−)-bicuculline was approximately 270% compared to the pre-injection control, and was reached within 60 min.

The combined administration of a maximally effective dose of (−)-bicuculline (10 μg) with oxotremorine-M (500 ng) produced a biphasic response (Fig. 7). During the first 60 min, the dopamine response failed to differ significantly from that obtained with either drug given alone [$F(2,12)=0.03$, $P=0.97$]. However, from 60 min after administration of both (−)-bicuculline and oxotremorine-M, levels of dopamine increased further [$F(2,12)=4.48$, $P=0.0352$]. Over this period the response was significantly greater than to either (−)-bicuculline [$F(1,12)=6.64$, $P=0.0243$], or oxotremorine-M [$F(1,12)=6.74$, $P=0.0234$].

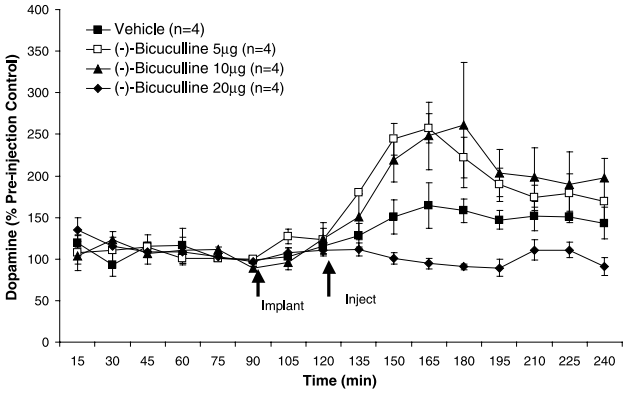

Fig. 6. Effect of ventral subiculum administration of (−)-bicuculline on extracellular dopamine in the nucleus accumbens. Data expressed as a percentage of a pre-injection control (mean ± S.E.M.). Injection needle implanted into ventral subiculum at first arrow; drugs delivered 30 min later at second arrow.

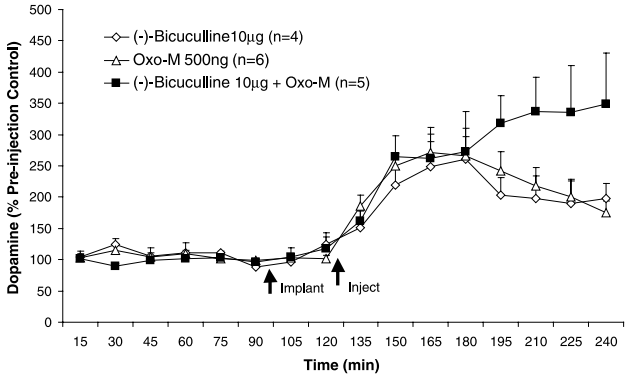

Fig. 7. Effect of the combined ventral subiculum administration of (−)-bicuculline (10 μg) and oxotremorine-M (500 ng) on extracellular dopamine in the nucleus accumbens, compared to either drug given alone. Data expressed as a percentage of a pre-injection control (mean ± S.E.M.). Injection needle implanted into ventral subiculum at first arrow; drugs delivered 30 min later at second arrow.

4. Discussion

Activation of the hippocampal projection to the nucleus accumbens, following microinjection of NMDA or carbachol into the ventral hippocampus or ventral subiculum has been reported to increase locomotor activity (Mogenson and Nielsen, 1984; Yang and Mogenson, 1987; Wu and Brudzynski, 1995; Brenner and Bardgett, 1998). The ability to prevent the behavioural response by systemic or local administration of glutamate or dopamine receptor antagonists indicated that the response was a consequence of an interaction between glutamate and dopamine systems, and to a consequent increase in levels of dopamine. Using in vivo microdialysis, a number of authors have now confirmed that extracellular levels of dopamine in the nucleus accumbens are increased after ventral hippocampal/ventral subiculum administration of NMDA (Brudzynski and Gibson, 1997; Legault and Wise, 1999; Legault et al., 2000; Mitchell et al., 2000b).

The present study shows that ventral subiculum administration of carbachol can also increase dopamine levels in the nucleus accumbens. In addition we show that the projection from the ventral subiculum is sensitive to muscarinic rather than nicotinic receptor stimulation. Firstly, the response to carbachol—a mixed nicotinic and muscarinic agonist—was prevented by co-administration with the muscarinic antagonist, atropine. Secondly, application of nicotine itself, or the α-7 nicotinic acetylcholine receptor agonist AR-R17779, failed to evoke a dopamine response. The α-7 nicotinic acetylcholine receptor subtype has been reported to exist in high amounts in the hippocampus, and has been implicated in the control of sensory motor gating (Leonard

et al., 2000). However, under these experimental conditions activation of α-7 nicotinic acetylcholine receptors in the ventral subiculum failed to alter the activity of the nucleus accumbens dopamine system.

Direct evidence for a sensitivity of the ventral subiculum to stimulation of muscarinic acetylcholine receptors was provided by the response to the broad-spectrum muscarinic receptor agonist, oxotremorine-M. Further studies utilising subtype-preferring agonists indicated the absence of a sensitivity of the ventral subiculum to muscarinic M_1 (and perhaps muscarinic M_3) receptor stimulation, but revealed an apparent sensitivity to the stimulation of muscarinic M_2/M_4 receptors. These findings were based on the observation that pilocarpine (M_1 and M_3 preferring; Lisa Broad, personal communication) and xanomeline (M_1 preferring; Sauerberg et al., 1992; Bymaster et al., 1994, 1997; Shannon et al., 1994) failed to evoke a dopamine response in the nucleus accumbens, whereas BuTAC, a partial agonist at muscarinic M_2 and M_4 receptors (Sauerberg et al., 1998; Shannon et al., 1999) produced a robust response. Interestingly the maximal response obtained with BuTAC (to approximately 169% of the pre-injection control) was lower than that obtained with the highest dose of oxotremorine-M tested. These effects may be consistent with BuTAC being a partial agonist, or that BuTAC activates only a sub-population of muscarinic receptors that underlie the response to oxotremorine-M. Interestingly, methoctramine, a muscarinic M_2/M_4 receptor antagonist (Dörje et al., 1991) significantly reduced the oxotremorine-M evoked response. These results suggest that M_2 and/or M_4 receptors can regulate the activity of the ventral subiculum. However, the inability of methoctramine to completely abolish the response to oxotremorine-M may imply a role for other muscarinic receptor subtype(s) as well.

Acetylcholine receptor stimulation of neurones within the hippocampal formation results in excitatory and inhibitory effects that are mediated mainly through the activation of muscarinic receptors (Rouse et al., 1999). In the subiculum, stimulation of acetylcholine receptors has been reported to increase neuronal excitability (Kawasaki et al., 1999). In these studies, application of carbachol to subicular neurones in vitro evoked a profound increase in intrinsic excitability, characterised by a reduction in burst-afterhyperpolarisations and the appearance of depolarising plateau potentials (Kawasaki et al., 1999). These effects of carbachol were reversed by application of atropine, implying activation of muscarinic receptors, however, the muscarinic receptor subtype mediating these events were not investigated. Nevertheless, it is likely that a sensitivity of subicular neurones to muscarinic receptor stimulation may have consequences for the output of the ventral subiculum in vivo, and such an action may underlie the neurochemical response reported here in the nucleus accumbens. A relationship between sensitivity of subicular neurones in vitro and functional activation in vivo has also been suggested to underlie the activity of the somatostatin receptor agonist seglitide (MK-678). In these studies MK-678, which induced burst-firing activity in subicular neurones in vitro (Greene and Mason, 1996), caused a long-lasting increase in extracellular dopamine in the ipsilateral nucleus accumbens in vivo (Mitchell et al., 2000a).

The experiments reported here also show a functional link between the ventral subiculum and nucleus accumbens, as measured by microdialysis. Interestingly, it has been argued that elevations in nucleus accumbens dopamine following ventral subiculum stimulation may not result entirely from a direct presynaptic mechanism in the nucleus accumbens. For instance, intra-subiculum administration of NMDA also increases dopamine efflux (Legault et al., 2000) and the number of spontaneously firing dopamine cells (Floresco et al., 2001) in the dopamine cell body, the ventral tegmental area. Moreover, the increase in dopamine release in the nucleus accumbens following ventral subiculum administration of NMDA was abolished by application of the glutamate receptor antagonist, kynurenic acid, in the ventral tegmental area (Legault and Wise, 2000), whilst the increase in activity of dopamine cells in the ventral tegmental area was prevented by local application of kynurenic acid in the nucleus accumbens (Floresco et al., 2001). A direct effect on dopamine efflux in the nucleus accumbens following stimulation of the ventral subiculum, however, cannot be excluded, as inhibiting NMDA receptors in the nucleus accumbens blocked the increase in dopamine efflux evoked by electrical stimulation of the ventral subiculum (Blaha et al., 1997; Taepavarapruk et al., 2000). Therefore it is likely that ventral subiculum modulation of dopamine in the nucleus accumbens may result from both a direct presynaptic mechanism in the nucleus accumbens and a polysynaptic mechanism involving the nucleus accumbens, ventral pallidum and ventral tegmental area.

Within the ventral subiculum itself, the precise neurocircuity underlying the muscarinic modulation of the ventral subiculum reported here is presently unknown. The data presented suggests that muscarinic M_2 and/or M_4 receptors may contribute to the effect of oxotremorine-M, but cannot account for the whole response. It is widely believed that muscarinic M_2 receptors are presynaptic autoreceptors and inhibit acetylcholine release (Zhang et al., 2002). Muscarinic M_4 receptors like M_2 receptors are coupled to G-proteins of the Go/Gi family and can also cause reduction in neuronal activity (Caulfield, 1993). Muscarinic M_4 receptors are expressed by medium spiny GABAergic neurones (Bernard et al., 1992; Hersch et al., 1994; Santiago and Potter, 2001), and muscarinic agonists can decrease GABA release (in the striatum; Marchi et al., 1990; Sugita et al., 1991). In the ventral subiculum, a reduction in GABA neurotransmission following local administration of the $GABA_A$ receptor antagonist (−)-bicuculline can also increase dopamine efflux in the nucleus accumbens, as reported previously (Mitchell et al., 2000b). Therefore, a muscarinic-receptor mediated reduction in GABA release (acting via $GABA_A$ receptors) in the ventral subiculum may

lead to an increase in burst firing activity and a net excitation of the projection to the nucleus accumbens. Studies to explore the mechanism of action of oxotremorine-M revealed that there did appear to be component to oxotremorine-M's effect that was dependent on a reduced GABA function. In combination with a maximally effective dose of (−)-bicuculline, the oxotremorine response was initially identical to either drug given alone. However, the response profile was biphasic, with a second component being additive with respects to both muscarinic receptor stimulation and $GABA_A$ receptor inhibition. From the response profile obtained with the combination of oxotremorine-M and (−)-bicuculline, it is tempting to suggest that two mechanisms underlie ventral subiculum stimulation by oxotremorine-M. The first, a muscarinic receptor mediated inhibition of GABA release, and the second, a muscarinic receptor mediated effect that is independent of GABA.

In summary, administration of carbachol into the ventral subiculum increased dopamine levels in the nucleus accumbens. The neurochemical response was not dissimilar to that obtained with local application of NMDA, and supports behavioural evidence that the increase in locomotor activity was due to an increase in dopamine release (Brenner and Bardgett, 1998). The ability to antagonise the response to carbachol with atropine suggested that the neurochemical response represented an interaction with muscarinic rather than nicotinic acetylcholine receptors. Activation of muscarinic receptors directly, following ventral subiculum application of oxotremorine-M, confirmed the sensitivity of the projection system to muscarinic acetylcholine receptor stimulation. Using selective muscarinic receptor agonists and antagonists, the ventral subiculum was insensitive to muscarinic M_1, but sensitive to muscarinic M_2 and/or M_4 receptor stimulation. The ability of methoctramine to partly reduce the oxotremorine-M-evoked response in the nucleus accumbens also suggested a contribution of muscarinic M_2/M_4 receptors in the regulation of ventral subiculum activity, but indicated that other muscarinic receptors may also be involved. Moreover, studies to understand the mechanism underlying the activity of oxotremorine-M in the ventral subiculum suggested two possible mechanisms that were dependent and independent of GABA neurotransmission.

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