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Presynaptic nicotinic receptors modulating dopamine release in the rat striatum

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Abstract

The modulation of striatal dopamine release by presynaptic nicotinic acetylcholine receptors is well documented for both synaptosomes and slices. Because the latter retain local anatomical integrity, we have compared [^{3}H]dopamine release evoked by the nicotinic receptor agonists (−)-nicotine and (±)-anatoxin-a from striatal synaptosome and slice preparations in parallel. At higher agonist concentrations, mecamylamine-sensitive [^{3}H]dopamine release was greater from slices, indicative of an additional component, and this increase was abolished by glutamate receptor antagonists. To begin to examine the localisation of specific nicotinic acetylcholine receptor subtypes in the striatum, immunogold electron microscopy was undertaken with the β2-specific monoclonal antibody 270. In striatal sections, gold particles were associated with symmetric synapses (dopaminergic) but were absent from asymmetric synapses (glutamatergic). Surface labelling of striatal synaptosomes with gold particles was also demonstrated. Taken together, these results are consistent with dopamine release mediated by β2-containing nicotinic acetylcholine receptors on dopamine terminals, while non-β2-containing nicotinic acetylcholine receptors may enhance dopamine release indirectly by releasing glutamate from neighbouring terminals.

Keywords: Nicotinic receptor, presynaptic; Glutamate receptor antagonist; Nicotine; Anatoxin-a; Immunogold electron microscopy; Synaptosome

1. Introduction

The widespread occurrence of nicotinic acetylcholine receptors at presynaptic locations in the central nervous system is well established (Wonnacott, 1997). The modulatory actions of presynaptic nicotinic acetylcholine receptors, by stimulating transmitter release or by enhancing synaptic efficacy (e.g., Gray et al., 1996), are considered to constitute a major role of neuronal nicotinic acetylcholine receptors (Role and Berg, 1996). The nicotinic modulation of transmitter release can be measured directly in vitro using neurochemical methods (Kaiser and Wonnacott, 1998) and dopamine release from striatal preparations has been the most widely studied example. Not only is the nicotinic stimulation of dopamine release robust, but it has merited study because of the role of dopamine in the rewarding and locomotor activating properties of nicotine. It is also pertinent to the therapeutic potential of nicotinic drugs for Parkinson's disease (Menzaghi et al., 1997).

Synaptosomes enable the direct modulation of nerve terminal function to be studied unambiguously, as anatomical connections are severed in the preparation and superfusion methodology is considered to prevent cross-talk between synaptosomes (Raiteri et al., 1974). The stimulation of [^{3}H]dopamine release from striatal synaptosomes by nicotinic receptor agonists has been widely reported, and shown to be dose dependent and inhibited by antagonists such as dihydroβerythroidine and mecamylamine, but not by α-bungarotoxin (Rapier et al., 1990; Grady et al., 1992; el-Bizri and Clarke, 1994; Soliakov et al., 1995). The nicotinic stimulation of [^{3}H]dopamine release is Ca^{2+} dependent, but is also blocked by Cd^{2+} and by antagonists of voltage-operated Ca^{2+} channels (Soliakov and Wonnacott, 1996), suggesting that the mechanism of triggering transmitter release involves local depolarisation and opening of Ca^{2+} channels. Recent evidence suggests that nicotinic acetylcholine receptors on striatal dopamine terminals are heterogeneous, comprising one population that contains α3 and β2 subunits (that is blocked by α-conotoxin MII)

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and a second population that is insensitive to this toxin, possibly containing α4 and β2 subunits (Kulak et al., 1997; Kaiser et al., 1998).

Slice preparations (prisms or minces) retain local anatomical integrity, enabling neuronal interactions to be examined. This may account for the differences in sensitivity to tetrodotoxin of nicotine-evoked [^{3}H]dopamine release from synaptosomes and slices (Marshall et al., 1996). The nicotinic stimulation of dopamine release from slices is dose-dependent (Sacaan et al., 1995). Dopamine release is subject to modulation by a diversity of transmitters via heteroreceptors present on dopamine terminals (Langer, 1997). In particular, there is considerable evidence that glutamate can elicit dopamine release, by acting at AMPA and NMDA receptors on dopamine terminals in the striatum (Cheramy et al., 1996). Moreover, locally applied nicotine can provoke glutamate release, measured by in vivo microdialysis (Toth et al., 1993). In vitro, electrophysiological studies have disclosed the ability of presynaptic nicotinic acetylcholine receptors to modulate glutamate release in rat hippocampus (Gray et al., 1996) and olfactory bulb (Alkondon and Albuquerque, 1995; Alkondon et al., 1996).

These various observations have prompted us to compare striatal synaptosomes and slices with respect to the release of [^{3}H]dopamine stimulated by nicotinic receptor agonists, and to assess the effects of ionotropic glutamate receptor antagonists on nicotinic acetylcholine receptor-evoked [^{3}H]dopamine release. This is coupled with an immunocytochemical study, at the electron microscope level, to begin to address the localisation of presynaptic nicotinic acetylcholine receptors in the rat striatum.

2. Materials and methods

2.1. Materials

[7,8-^{3}H]Dopamine (specific activity, 1.78 Tbq/mmol) was purchased from Amersham International, Amersham, Bucks, UK. Pargyline and (−)-nicotine ditartrate were purchased from Sigma, Poole, Dorset, UK. Nomifensine was obtained from R.B.I., Poole, Dorset, UK. and (±)anatoxin-a, 6,7-dinitroquinoxaline-2,3-dione (DNQX), and kynurenic acid were obtained from Tocris Cookson, Bristol, UK. Rat monoclonal antibody to nicotinic acetylcholine receptor β2 subunit (monoclonal antibody 270) was kindly donated by Prof. J. Lindstrom, University of Pennsylvania, USA. Gold conjugated secondary antibodies were purchased from British Biocell, Cardiff, UK. All other chemicals used were of analytical grade and obtained from standard commercial suppliers.

2.2. Preparation of striatal synaptosomes and slices

Male Sprague–Dawley rats (240–280 g, University of Bath breeding colony) were killed by cervical dislocation, decapitated and the brain removed. Striata from two rats were rapidly dissected on ice (180–240 mg of wet weight tissue per rat) and used for the preparation of either synaptosomes or slices.

P2 synaptosomes were prepared as previously described (Soliakov et al., 1995). Percoll purified synaptosomes (fraction 4) were prepared according to the procedure of Dunkley et al. (1988) as previously described by Soliakov and Wonnacott (1996). Synaptosomes were loaded with [^{3}H]dopamine (0.1 μM, 0.185 MBq/ml) and superfused in open chambers.

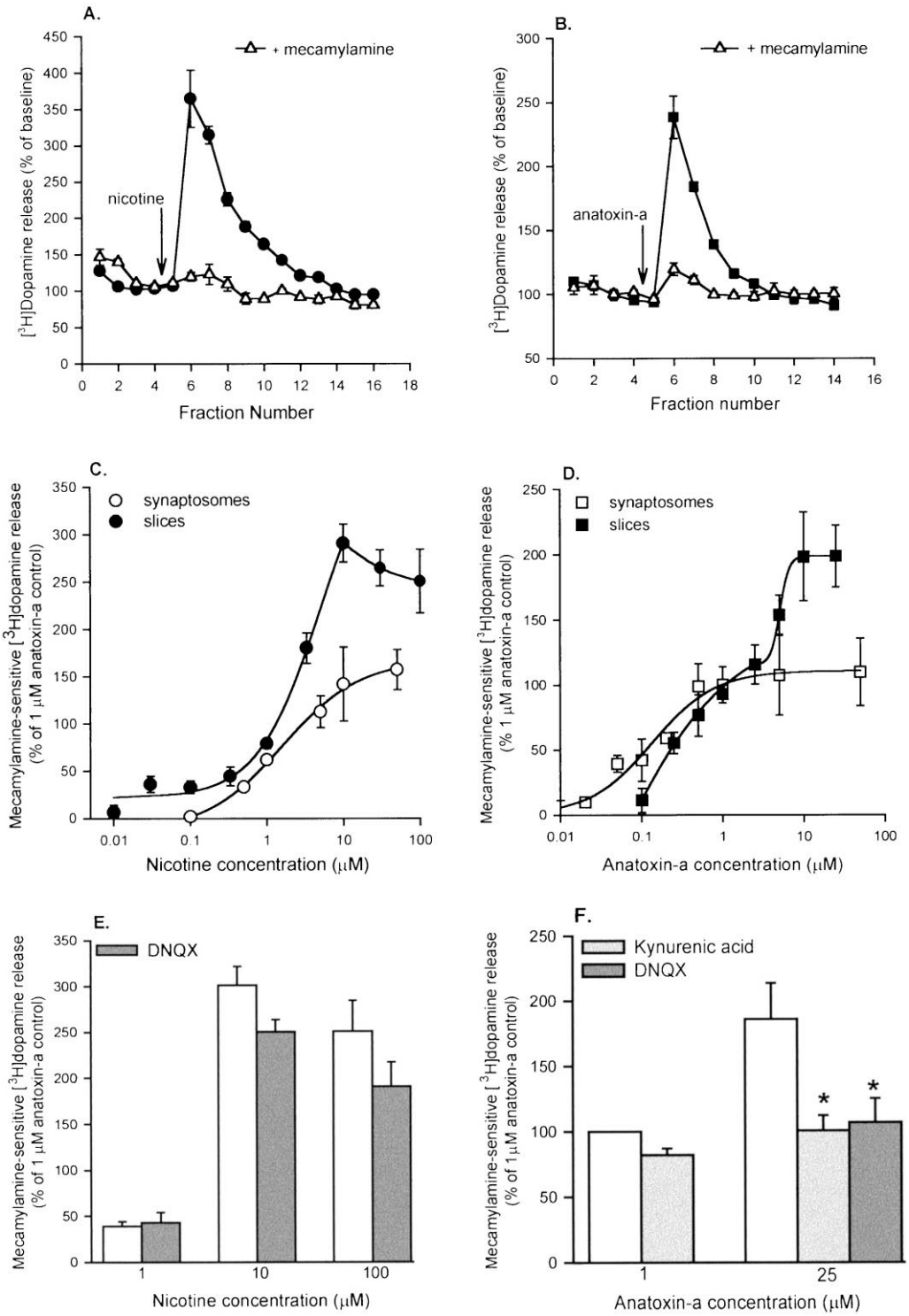
Striatal slices (0.25 mm prisms) were prepared as previously described by Marshall et al. (1996) using a McIlwain tissue chopper. The tissue slices were washed twice with Kreb's buffer and incubated with [^{3}H]dopamine (0.1 μM, 0.185 MBq) for 15 min, followed by washing twice in Kreb's buffer. Aliquots (200 μl, 40–50 mg tissue) of the slice suspension were loaded into 12 closed superfusion chambers. All superfusion experiments were performed in Kreb's bicarbonate buffer of the following composition: 118 mM NaCl, 2.4 mM KCl, 2.4 mM $CaCl_2.2H_2O$, 1.2

Fig. 1. Nicotine and anatoxin-a-evoked [^{3}H]dopamine release from striatal synaptosomes and slices. (A, B) Representative superfusion profiles showing [^{3}H]dopamine release from rat striatal slices. Slices were loaded with [^{3}H]dopamine and superfused as described in Materials and methods; 2 min (1 ml) fractions were collected. Antagonist (10 μM mecamylamine; ⊠) was added to the perfusing buffer 10 min prior to stimulation for 40 s with either 10 μM (−)-nicotine (●; A) or 25 μM (±)-anatoxin-a (■; B). Release is normalised to the baseline and expressed as a percentage. Error bars indicate the S.E.M. from at least three separate experiments, each consisting of two or more replicate chambers. (C, D) Dose–response curves for [^{3}H]dopamine release evoked by (−)-nicotine and (±)-anatoxin-a in rat striatal synaptosome and slices. Release of [^{3}H]dopamine in response to (−)-nicotine (●, ○; C) or (±)-anatoxin-a (■, □; D) was determined over a range of concentrations in preparations of rat striatal synaptosomes (open symbols) and slices (filled symbols). Responses determined in parallel in the presence of mecamylamine were subtracted. Specific [^{3}H]dopamine release, calculated as a fraction of total [^{3}H]dopamine present at the time of stimulation, is expressed as a percentage above basal release. Error bars indicate S.E.M for at least three separate experiments. Data are fitted to a one-site Hill equation, except in the case of anatoxin-a-evoked [^{3}H]dopamine release in striatal slices (■), where the data are fitted to a two-site model. EC_{50} values for nicotine and anatoxin-a were determined from the curve fit, and values are given in Table 1. (E, F) Comparison of the effects of glutamate antagonists on (−)-nicotine and (±)anatoxin-a-evoked [^{3}H]dopamine release from rat striatal slices. Release of [^{3}H]dopamine evoked by (−)-nicotine (1, 10 and 100 μM; E) was examined in the presence and absence of the AMPA/kainate receptor selective antagonist DNQX (100 μM); antagonist was added to the perfusing buffer 10 min prior to stimulation with agonist. Release of [^{3}H]dopamine evoked by (±)-anatoxin-a (1 and 25 μM; F). It was examined in the presence and absence of the general glutamate receptor antagonist kynurenic acid (500 μM), as well as DNQX (100 μM) at the higher concentration of anatoxin-a. Mecamylamine-insensitive release was subtracted and results are expressed as a percentage of the response to 1 μM anatoxin-a. Error bars indicate the S.E.M. from at least three separate experiments.

mM $MgSO_4.7H_2O$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ and 10 mM glucose buffered to pH 7.4, saturated with 95% O_2/5% CO_2 and supplemented with 1 mM ascorbic acid and 8 μM pargyline to prevent dopamine degradation (0.5 μM nomifensine was added to the buffer after loading with [^{3}H]dopamine to prevent dopamine re-uptake).

2.3. Superfusion of synaptosomes and slices

Superfusion of synaptosomes (open chambers) or slices (closed chambers) was performed as previously described (Marshall et al., 1996) in a Brandel superfusion apparatus using Kreb's bicarbonate buffer at a flow rate of 0.5



ml/min and a temperature of 37°C; 2-min fractions were collected. After a 30-min wash out period, agonist was applied for 40 s, separated from the bulk buffer flow by 10 s air bubbles. Where antagonists were examined, they were introduced into the buffer 10 min before agonist stimulation and remained throughout the remainder of the experiment. Fractions of perfusate were counted for radioactivity in a Packard Tricarb 1600 liquid scintillation counter (counting efficiency 48%).

2.4. Superfusion data analysis

The baseline was derived by fitting the following double exponential decay equation to the data, using the program SigmaPlot (v 2.0) for Windows:

$$y = ae^{-bx} + ce^{-dx}$$

where a, b, c, d are the curve parameters and x is the fraction number. Evoked [^{3}H]DA release was calculated as the area under the peak, after subtraction of the baseline, and normalised to the total radioactivity present at the time of agonist stimulation. Data are presented as either percent above basal release or percent of corresponding controls, run in parallel. For comparison between different experiments, superfusion profiles were normalised as percentages of fitted baselines. Values are the mean ± S.E.M. of the number of independent experiments carried out, each experiment consisting of two or three replicate chambers for each condition.

2.5. Post-embedding immunocytochemistry

Lowicryl™ (Agar Scientific, Stanstead, UK) resin-embedded rat striatal blocks were kindly donated by Prof. J.P. Bolam, University of Oxford, UK. Post-embedding immunocytochemistry was performed as described by Nusser et al. (1995). Briefly, 60–80 nm sections were obtained using an ultramicrotome (Ultracut E™, Leica) and collected on gold mesh grids. Once dry, the grids were dipped in sodium ethanolate for 2–3 s to etch the surface of the resin and then rinsed in several changes of distilled water. The grids were then washed 2 × 5 min in Tris-buffered saline (TBS; 50 mM Tris, pH 7.4, 0.9% NaCl) and incubated in 2% (w/v) human serum albumin (HSA) in TBS + 0.01% Triton X-100 (TBST) for 30 min to block non-specific binding sites. The grids were incubated in monoclonal antibody 270, at 5 μg/ml in 2% HSA/TBST, overnight.

Following 3 × 10 min washes in TBS and 1 × 10 min wash in 2% HSA/TBST, the grids were incubated in the secondary antibody (10 nm gold-conjugated anti-rat IgG), diluted 1:20 in 2% HSA/TBST + 0.05% (v/v) polyethyleneglycol. The grids were then washed 3 × 10 min in TBS and 1 × 10 min in 0.1 M phosphate buffer (PB) before being post-fixed in 2% (w/v) glutaraldehyde in 0.1 M PB for 2 min. The grids were then rinsed in several changes of distilled water and dried. After counterstaining with uranyl acetate and lead citrate according to standard protocols (Reynolds, 1963), the grids were viewed in the transmission electron microscope (1200EX, Jeol) at 80 kV.

Table 1
EC_{50} values for the stimulation of [^{3}H]dopamine release from striatal synaptosomes and slices by nicotine and anatoxin-a

Agonist	EC_{50} values (μM)	
	Synaptosomes	Slices
(−)-Nicotine	1.6 ± 0.3	5.0 ± 3.6
(±)-Anatoxin-a	0.13 ± 0.03	0.24 ± 0.04; 5.1 ± 0.3

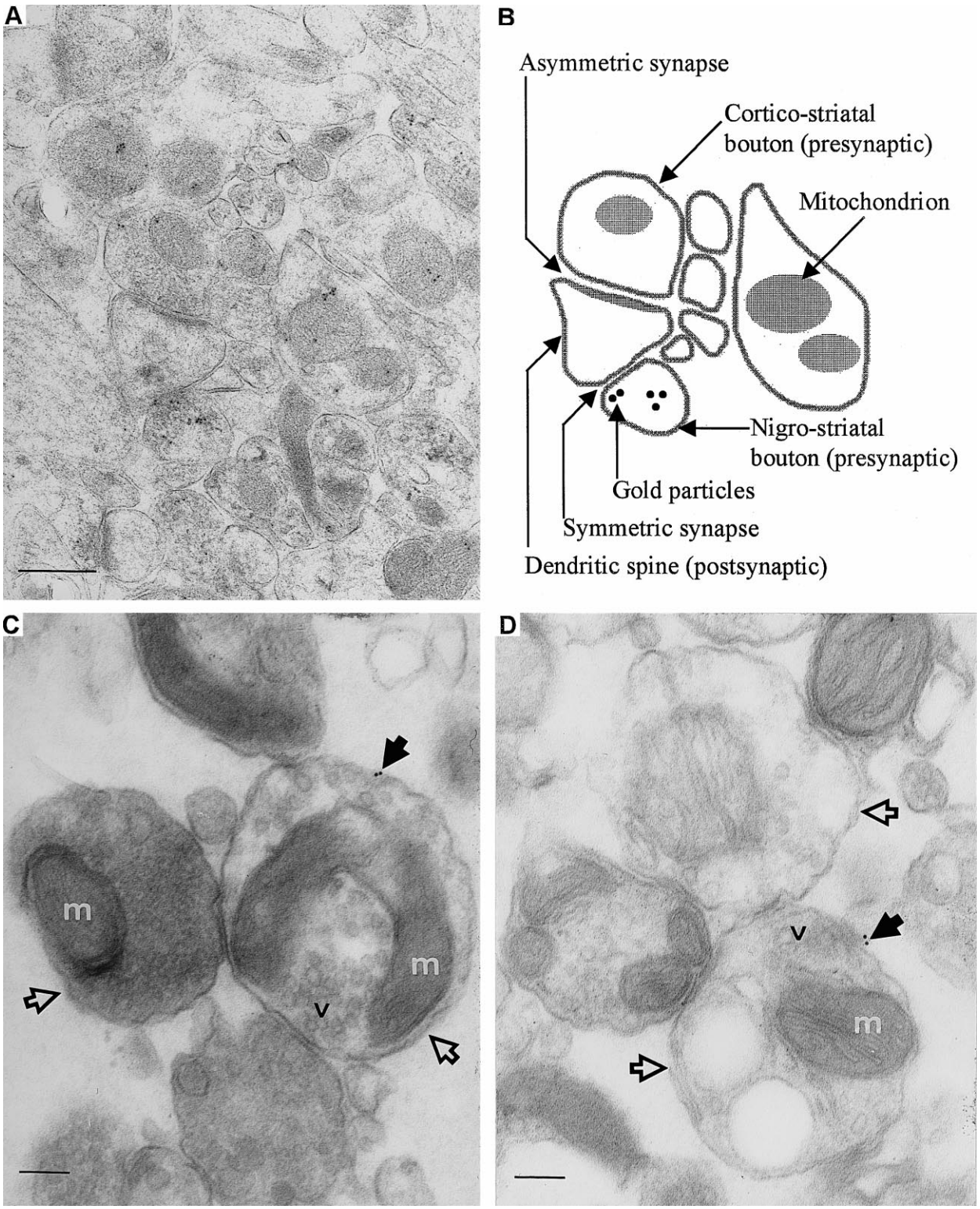
2.6. Synaptosome immunocytochemistry

Percoll-purified striatal synaptosomes were fixed in 3% (w/v) paraformaldehyde + 0.1% (w/v) glutaraldehyde in Kreb's buffer for 1 h at 4°C. The synaptosomes were then pelleted by centrifuging at 3000 rpm for 1 min in a bench-top centrifuge (MicroCentaur™, MSE) and washed (3 × 5 min) in ice cold Kreb's buffer followed by 1 × 5 min in ice cold phosphate-buffered saline (PBS; 10 mM PB, 0.9% NaCl). The synaptosomes were next incubated in 0.5% (v/v) normal rabbit serum in PBS (NRS/PBS) for 1 h to block non-specific binding sites and then in monoclonal antibody 270, diluted to 5 μg/ml in 0.5% NRS/PBS, overnight at 4°C.

The synaptosomes were washed 3 × 10 min in PBS and then incubated in secondary antibody (10 nm gold-conjugated anti-rat IgG), diluted 1:200 in 0.5% NRS/PBS, for

Fig. 2. Immunolocalisation of nicotinic acetylcholine receptor β2 subunit in rat striatal sections and synaptosomes. Panels A and B: Post-embedding nicotinic acetylcholine receptor β2 subunit immunocytochemistry in the adult rat striatum using monoclonal antibody 270. The micrograph (panel A) and associated trace diagram (panel B) show nigro-striatal and cortico-striatal boutons forming symmetric and asymmetric synapses, respectively, on a dendritic spine within the striatum. β2 subunit immunoreactivity, indicated by the presence of 10-nm gold particles, is predominantly present in the cytoplasm and on the surface of the nigro-striatal bouton, close to the symmetric synapse. The gold label associated with mitochondria is due to non-specific binding of the secondary antibody, as it was not eliminated by omission of the primary antibody, in contrast to the bouton labelling. Scale bar = 200 nm. Panels C and D: Nicotinic acetylcholine receptor β2 subunit immunolabelling of adult rat striatal synaptosomes. Striatal synaptosomes were purified on Percoll gradients and subjected to post-embedding imunolabelling, with monoclonal antibody 270 and gold conjugated second antibody, as described in Materials and methods. In both panels, β2 subunit immunoreactivity, indicated by the presence of 10-nm gold particles, is evident on the surface of a subpopulation of synaptosomes, indicating the presence of membrane-bound receptor subunits. Open arrow = synaptosome; Filled arrow = immunogold particles; m = mitochondrion; v = synaptic vesicles. Scale bar = 100 nm.

1 h. Following 5 × 10 min washes in PBS, the synaptosomes were post-fixed in 1% (w/v) glutaraldehyde in PBS for 10 min and then washed 2 × 10 min in PBS and 1 × 10 min in PB. The synaptosomes were further post-fixed in 1% (w/v) osmium tetroxide in 0.1 M PB for 30 min and then washed 3 × 15 min in 0.1 M PB followed by 2 × 15 min in 50% ethanol. The synaptosome pellets were then dehydrated through an increasing ethanol series into dry absolute ethanol, rinsed in propylene oxide, and covered in freshly prepared acrylic resin (Durcopan™, Fluka, Gillingham, UK) overnight. The resin was then replaced with fresh resin and the pellets cured at 60°C for 48 h.



After curing, 60 nm ultrathin sections were taken from the pellet blocks, using an ultramicrotome (Ultracut E™, Leica), counterstained in uranyl acetate and lead citrate according to standard protocols (Reynolds, 1963), and viewed in the transmission electron microscope (1200EX, Jeol) at 80 kV.

3. Results

3.1. [^{3}H]Dopamine release

(−)-Nicotine and (±)-anatoxin-a were compared for their abilities to release [^{3}H]dopamine from rat striatal synaptosomes and slices (Fig. 1). A 40-s pulse of agonist elicited a peak of radioactivity above basal release that was almost totally abolished in the presence of 10 μM mecamylamine (Fig. 1A,B). A range of concentrations of the agonists was tested in the presence and absence of mecamylamine, and the mecamylamine-insensitive component of release was subtracted to yield dose–response curves for specific nicotinic acetylcholine receptor-stimulated [^{3}H]dopamine release (Fig. 1C,D). Data for both synaptosomes and slices are expressed as a percentage of the response to 1 μM anatoxin-a, in order to compare the dose–response relationships between striatal preparations; EC_{50} values are given in Table 1. Note that the scales of Fig. 1C and D differ, reflecting the greater efficacy of nicotine in both synaptosome and slice preparations. It is apparent that for both nicotine and anatoxin-a, the dose response curves for synaptosomes and slices agree reasonably well at low agonist concentrations, whereas at higher concentrations the two curves deviate with relatively more release observed with slice preparations.

One interpretation of these data is that an additional component contributes to [^{3}H]dopamine release from slices at higher agonist concentrations. Indeed, the dose response curve for anatoxin-a-evoked [^{3}H]dopamine release from slices is best fitted by a two-site Hill equation (Fig. 1D), giving EC_{50} values of 0.24 and 5 μM, consistent with the involvement of a lower affinity component that is absent in the synaptosome responses. A candidate mechanism to account for this additional component is an indirect nicotinic acetylcholine receptor action mediated by glutamate release. This hypothesis was examined by investigating the effects of the glutamate receptor antagonists kynurenic acid and 6,7-dinitroquinoxaline-2,3-dione (DNQX) on nicotine- and anatoxin-a-evoked [^{3}H]dopamine release from slices (Fig. 1E,F). At the lower agonist concentrations examined (1 μM nicotine and anatoxin-a), the glutamate receptor antagonists had little effect. At higher agonist concentrations (10, 100 μM nicotine; 25 μM anatoxin-a) the antagonists decreased [^{3}H]dopamine release by about 20% in the case of nicotine and 50% in the case of anatoxin-a. In contrast, kynurenic acid and DNQX had no significant effect on nicotinic receptor agonist-evoked [^{3}H]dopamine release from synaptosomes (data not shown).

3.2. Immunogold electron microscopy

We have initiated an examination of the subcellular localisation of nicotinic acetylcholine receptor subtypes in the striatum by investigating the distribution of β2 subunits using monoclonal antibody 270, a rat monoclonal antibody directed against an extracellular epitope of the subunit. At the light microscope level, monoclonal antibody 270 labelled cell bodies and processes in the substantia nigra pars compacta, consistent with expression of β2 in dopaminergic neurones. However, at this level of magnification no immunostaining was detectable in the striatum (data not shown). To resolve the labelling of β2 subunits on striatal terminals, resin-embedded tissue blocks were subjected to post-embedding immunocytochemistry (after Baude et al., 1993), by incubating ultrathin (80 nm) sections with monoclonal antibody 270 followed by a 10 nm gold-conjugated second antibody. A typical example of the results of this technique is shown in Fig. 2A. Gold particles are observed in the cytoplasm of an axon terminal making a symmetric synapse with the neck of a dendritic spine (Fig. 2B); these characteristics define dopaminergic terminals in the striatum (Smith and Bolam, 1990). In contrast, a terminal densely packed with synaptic vesicles and making an asymmetric synapse onto the head of the same dendritic spine (characteristics typical of a corticostriatal glutamatergic afferent) is devoid of gold particles. Other structures, such as mitochondria, are labelled both in the presence and absence of monoclonal antibody 270, suggesting non-specific binding of the secondary antibody to these features.

To complement the post-embedding tissue study, striatal synaptosome preparations were surface labelled with monoclonal antibody 270, using a novel pre-embedding technique (Fig. 2C,D). For this, Percol-purified synaptosomes were first aldehyde fixed and then immunolabelled prior to embedding in resin and sectioning. The results show gold particles associated with the plasma membrane of a proportion of synaptosomes present. Pairs of gold particles are seen together, suggesting that the nicotinic acetylcholine receptor may be clustered. In the absence of monoclonal antibody 270, no gold particles were observed (not shown).

4. Discussion

The dose-dependant release of [^{3}H]dopamine from striatal slices and synaptosomes is well documented (Grady et al., 1992; el-Bizri and Clarke, 1994; Sacaan et al., 1995) but these preparations have rarely been compared in the same laboratory. Here we have demonstrated that higher concentrations of both nicotine and anatoxin-a evoke a relatively greater response from slices than from synapto-

somes (Fig. 1C,D). As slices retain local anatomical integrity, they afford an opportunity for additional modulation of [^{3}H]dopamine release by other transmitters released by nicotinic acetylcholine receptor stimulation of other terminals. Glutamate and the glutamate receptor agonists AMPA and kainate have been shown to release dopamine from striatal synaptosomes (Roberts and Anderson, 1979; Cheramy et al., 1994) and slices in vitro (Jin, 1997) and in vivo (Jedema and Moghddam, 1996) The ability of the non-selective ionotropic glutamate receptor antagonist kynurenic acid and the AMPA/kainate receptor antagonist DNQX to decrease [^{3}H]dopamine release evoked by higher concentrations of nicotine and anatoxin-a (Fig. 1E,F) supports a contribution from glutamate in this release process. The failure of these antagonists to inhibit [^{3}H]dopamine release from striatal synaptosomes argues against a non-specific action (arising from blockade of the nicotinic acetylcholine receptor channel for example). The concentrations used (500 μM for kynurenic acid, 100 μM for DNQX) are sufficient to fully inhibit glutamate receptors (Jin and Fredholm, 1997), suggesting that glutamate accounts for about 20% and 50% of nicotine and anatoxin-a-evoked [^{3}H]dopamine release, respectively. The different proportions may reflect the greater efficacy of nicotine in eliciting [^{3}H]dopamine release from both synaptosomes and slices, attributed to its greater efficacy at α4β2 nicotinic acetylcholine receptor dopamine terminals (Sharples et al., submitted).

Another approach in investigating the relationship between nicotinic acetylcholine receptors and transmitter systems in the striatum is to use immunocytochemistry at the electron microscope level. For this initial study, we have examined the localisation of the β2 subunit, using monoclonal antibody 270. This subunit was chosen because pharmacological evidence implicates β2-containing nicotinic acetylcholine receptors in the modulation of [^{3}H]dopamine release from striatal synaptosomes (Kulak et al., 1997; Kaiser et al., 1998) and this is supported by the absence of nicotine-evoked dopamine release in β2 null mutant mice (Grady et al., 1998; Picciotto et al., 1998) Moreover, monoclonal antibody 270 is a well-characterised antibody (Whiting and Lindstrom, 1987) that has proven useful for immunocytochemistry at the light microscope level in the rat brain (Swanson et al., 1987). Utilising post-embedding immunocytochemistry at the electron microscope level, analysis of labelled sections reveals that β2 immunoreactivity is predominantly found on axon terminals forming symmetric synapses with dendritic spines in the striatum (Fig. 2A). This pattern of staining is not seen when monoclonal antibody 270 is omitted from the staining protocol, suggesting that gold labelling is specific for the β2 subunit. These results suggest that β2 is present on dopamine terminals (symmetric synapses) but not on glutamate terminals (asymmetric synapses). Co-localisation of the β2 subunit with definitive markers, such as tyrosine hydroxylase, will be necessary to confirm this association with dopaminergic axons, and these experiments are currently underway.

In addition to the post-embedding tissue labelling of the β2 subunit, we have devised a method to surface label striatal synaptosomes with monoclonal antibody 270. This is essentially a pre-embedding technique, as the synaptosomes are immunolabelled prior to embedding in resin and sectioning for the electron microscope. This approach is feasible with monoclonal antibody 270 because it recognises an extracellular epitope on the β2 subunit. The electron micrographs clearly show that a proportion of striatal synaptosomes have gold particles bound to their surface, consistent with the presence of nicotinic acetylcholine receptors containing the β2 subunit. To the best of our knowledge, this is the first demonstration of successful immunogold labelling of a synaptosome preparation. From the morphology, it is not possible to infer the transmitter specificity of the gold-labelled synaptosomes. Dual labelling (of receptor subunit and transmitter marker or co-localisation studies with antibodies against two nicotinic acetylcholine receptor subunits), with different size gold particles for each antigen, will require post-embedding immunocytochemistry.

The data described here provide initial evidence that nicotinic receptor agonist-evoked glutamate release may in turn enhance dopamine release from striatal slices. The juxtaposition of glutamate and dopamine terminals around dendritic spines (Fig. 2A,B; Smith and Bolam, 1990) would facilitate such an interaction. The immunolocalisation of the β2 subunit is consistent with its association with dopamine terminals, which is compatible with pharmacological evidence for its participation in nicotinic heteroreceptors on striatal dopamine terminals (Kulak et al., 1997; Kaiser et al., 1998). Its apparent absence from glutamate terminals suggests that another nicotinic acetylcholine receptor subtype is responsible for glutamate release in the striatum. To date, most evidence for nicotinic acetylcholine receptor-mediated glutamate release involves α7-containing nicotinic acetylcholine receptors (McGehee et al., 1995; Alkondon et al., 1996; Gray et al., 1996) and this would be consistent with the contribution of the presumed glutamate component only at higher agonist concentrations, as α7 receptors are less sensitive to agonists than other subtypes (Alkondon and Albuquerque, 1995; Role and Berg, 1996). Further pharmacological analysis is needed to verify the participation of α7 nicotinic acetylcholine receptors in striatal dopamine release. Its involvement might be pertinent to mechanisms of motor control and nicotine dependence.

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