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Nicotinic receptors and Parkinson's disease

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Abstract

Accumulating evidence indicates that nicotinic receptors play a role in basal ganglia function. Furthermore, nicotine administration may be neuroprotective in animal models of nigrostriatal degeneration, while cigarette smoking is inversely correlated with Parkinson's disease. Because nicotinic receptors are decreased in Parkinson's disease, these observations may suggest that nicotinic agonists are beneficial in this disorder. We used two model systems to investigate this possibility. One involved non-human primates, which represent a good model because their neuroanatomical organization resembles that of man and nigrostriatal degeneration leads to biochemical and behavioral deficits similar to Parkinson's disease. To identify the subunits that comprise basal ganglia nicotinic receptors, we investigated $\alpha 4$, $\alpha 6$, $\alpha 7$, $\beta 2$, $\beta 3$ and $\beta 4$ transcript distribution in monkey substantia nigra. All mRNAs were expressed with a selective alteration in some transcripts after 1-methyl-4-phenyl-1,2,3,6-tetrahydropteridine (MPTP) induced nigrostriatal degeneration. As an approach to evaluate neuroprotective effects of nicotine against nigral neuron damage, we used mesencephalic neurons in culture, treated with a selective dopaminergic neurotoxin. The results show that nicotine pretreatment protected against dopaminergic nigral neural degeneration. These data suggest that nicotinic receptor ligands may be useful in Parkinson's disease therapy.

Keywords: Nicotinic receptor; Parkinson's disease; MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropteridine); (Monkey); Hybridization, in situ; Neuroprotection

1. Introduction

Nicotinic receptor stimulation plays an important role in a variety of central nervous system (CNS) functions including locomotor activity, cognition, addiction, reinforcement, affect, anxiety, as well as others. Of relevance to our studies are the effects of nicotine on locomotion, which appear to be mediated through an interaction with different nicotinic receptor subtypes in the basal ganglia (Stolerman et al., 1973; Clarke and Kumar, 1983; Balfour and Fagerstrom, 1996). Not only does nicotine modulate activity under physiological conditions, but it also ameliorates locomotor deficits observed after nigrostriatal degeneration in both animals and man. In 6-hydroxydopamine-treated rodents, nicotine administration modulates turning behavior (Lapin et al., 1987; Janson et al., 1988), while low-dose administration of nicotine or a nicotinic agonist, which have no significant effects on their own, enhances L-dopa-stimulated locomotor activity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropteridine (MPTP)-induced parkinsonian monkeys (Schneider et al., 1998a,b; Domino et al., 1999). Smoking or nicotine patch/gum usage has also been reported to reduce the tremor, rigidity, bradykinesia and gait disturbances observed in Parkinson's disease (Ishikawa and Miyatake, 1993; Fagerstrom et al., 1994), a debilitating movement disorder that occurs in approximately 1% of the population over 50 years of age (Lang and Lozano, 1998a,b). This reduction in Parkinson's disease symptomatology is observed shortly after the initiation of smoking, lasts approximately 10–30 min and appears more prominent after smoking than after chewing nicotine gum (Ishikawa and Miyatake, 1993; Fagerstrom et al., 1994; Clemens et al., 1995). The mechanism of the beneficial effect of nicotine after nigrostriatal degeneration may relate to the stimulation of nicotinic receptors. Firing of neurons in the zona compacta of the substantia nigra is accelerated after iontophoretic application of nicotine or after its subcutaneous injection (Lichtensteiger et al., 1982; Clarke et al., 1985), with a subsequent release in dopamine as well as other neurotransmitters (Rapier et al., 1988,

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1990; Grady et al., 1992, 1994; Balfour and Fagerstrom, 1996). Recent evidence suggests that synaptic α4-containing nicotinic receptors may be involved (Sorenson et al., 1998; Arroyo-Jiminez et al., 1999).

In addition to an effect on movement, nicotinic receptor stimulation also appears to play a pivotal role in cognition, which may be of relevance to the dementia observed in Parkinson's disease. Disruption of cholinergic transmission may cause a marked impairment of memory and learning (Newhouse et al., 1995; Balfour and Fagerstrom, 1996; Felix and Levin, 1997) while nicotine treatment improves performance in a number of tests of learning and memory in a range of behavioral models in rodents (Balfour and Fagerstrom, 1996). Moreover, nicotinic acetylcholine receptor agonists improve cognition in chronic MPTP-treated monkeys with and without motor disability (Schneider et al., 1998a). Nicotine administration to man also appears to improve cognition (Newhouse et al., 1995; Rusted et al., 1995), and may be of benefit to Parkinsonian patients (Fagerstrom et al., 1994).

Nicotine not only stimulates CNS function, but accumulating studies also indicate that nicotine and/or cigarette smoking may exert a protective action against neuronal degeneration. Epidemiological studies conducted over a 40-year period report a decreased incidence of Parkinson's disease among tobacco users. This relationship between disease protection and smoking is reproducible, of a generally consistent magnitude, exhibits a dose response and does not appear related to selective mortality (Baron, 1986, 1996; Morens et al., 1995; Balfour and Fagerstrom, 1996; Riggs, 1996; Hellenbrand et al., 1997). A knowledge of the component in cigarette smoke, which protects against Parkinson's disease, is important because it may provide insight into the mechanisms of degeneration and lead to treatment and/or preventive therapies. One hypothesis is that nicotine exerts a neuroprotective action. Chronic nicotine exposure attenuates nigrostriatal degeneration in partially hemitransected rats (Janson et al., 1988, 1991; Fuxe et al., 1990; Grenhoff et al., 1991), while nicotine/smoke exposure to mice protects against MPTP-induced nigrostriatal degeneration (Carr and Rowell, 1990; Shahi et al., 1991; Janson et al., 1992, but see also Perry et al., 1987; Serchen et al., 1987; Behmand and Harik, 1992; Hadjiconstantinou et al., 1994). In addition, experiments with cultured neuronal cells show that nicotine mediates a neuroprotective effect against toxin-induced cytotoxicity (Akaike et al., 1994; Kihara et al., 1997; Li et al., 1999).

A question that arises is the status of nicotinic receptors in Parkinson's disease, which occurs as a result of degeneration of the nigrostriatal pathway (Lang and Lozano, 1998a,b). Receptor binding studies, using [^{3}H]nicotine, have been done to assess the integrity of the nicotinic acetylcholine receptor population in Parkinson's disease. Declines of up to 50% have been demonstrated in frontal and temporal cortex and hippocampus, regions that are associated with memory and learning (Rinne et al., 1991; Aubert et al., 1992; Lange et al., 1993; Perry et al., 1995). Decreases have also been observed in the striatum in some studies (Aubert et al., 1992) but not others (Lange et al., 1993). The specific nicotinic receptor subtypes involved remain to be elucidated.

These findings, showing a loss of nicotinic receptors in the basal ganglia after nigrostriatal damage, suggest that administration of nicotinic receptor ligands have potential as therapeutic agents in Parkinson's disease. However, nicotine administration activates multiple nicotinic receptors in the nervous system. To date, 11 neuronal nicotinic receptor subunits genes have been identified, including α2 to α9 which encode ligand binding subunits and β2 to β4 which may represent structural subunits (Albuquerque et al., 1996; Lindstrom et al., 1996; Role and Berg, 1996; Wonnacott, 1997; Changeux et al., 1998). Receptors containing some combination of α2 to α6 and β2 to β4 subunits may form pentameric channels in the basal ganglia to comprise the neuronal nicotinic receptors (Albuquerque et al., 1996; Lindstrom et al., 1996; Role and Berg, 1996; Wonnacott, 1997; Changeux et al., 1998). These have been identified in both rodent and human striatum and substantia nigra. However, the radiolabelled agonists currently available do not readily distinguish between these different nicotinic receptor subtypes (Martino-Barrows and Kellar, 1987; Anderson and Arneric, 1994). To develop a selective therapy, it is essential to determine the specific nicotinic receptor subtype(s) that are altered after nigrostriatal damage. One of the objectives of our work was to identify changes in the different nicotinic receptor subtypes in MPTP-treated monkeys, an animal model that closely mimics Parkinson's disease. In our initial studies, we investigated the distribution of nicotinic receptor subunit mRNAs because receptor subtype identification per se is presently limited by a lack of subtype specific ligands. Another approach we used to investigate an involvement of nicotinic receptors in nigrostriatal degeneration included experiments to determine the neuroprotective potential of nicotine against dopaminergic neurotoxicity in mesencephalic cells in culture.

2. Materials and methods

2.1. The non-human primate model

2.1.1. Animals

Squirrel monkeys (*Saimiri sciureus*) weighing 0.7–1.0 kg, were housed separately using a 13-h light–11-h dark cycle. The animals were quarantined and tested according to standard veterinary practice. Before saline or MPTP (2 mg/kg) treatment, all monkeys underwent an initial testing period to evaluate baseline locomotor activity using a computerized movement monitor cage containing an

array of infrared sensors (Irwin et al., 1990; Alexander et al., 1991). This was repeated 2 1/2 weeks after MPTP or saline treatment. The monkeys were euthanized 4 weeks after injection with MPTP in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. All procedures used in this study conform to the NIH Guide for the Care and Use of Laboratory Animals.

2.1.2. Tissue preparation and assays

The brains were removed and cut into 6-mm thick blocks which were frozen in isopentane on dry ice. For catecholamine determinations, tissue punches (10–20 mg tissue each) were taken from the caudate and putamen. Dopamine and homovanillic acid (HVA) were extracted into 0.4 N perchloric acid and determined using HPLC coupled to electrochemical detection (Kilpatrick et al., 1986). For in situ hybridization, the blocks were cut into 20-μm thick sections, thaw-mounted onto poly-L-lysine-coated slides and stored at −80°C. The sections were fixed for in situ hybridization as previously described (Wada et al., 1989; Marks et al., 1992; Quik et al., 1998, 1999). cRNA probes were prepared to sequences encoding portions of the large cytoplasmic loop of the different human nicotinic receptor subunit cDNAs: α4, 1033–1557; α6, 1024–1353; α7, 949–1377; β2, 1058–1344; β3, 973–1243; β4, 1000–1338 (Quik et al., 1998, 1999). α-[^{35}S]UTP labeled probes were prepared using an in vitro Transcription Kit from Promega. In situ hybridization was done as described (Wada et al., 1989; Marks et al., 1992; Quik et al., 1998, 1999) using ^{35}S-radiolabelled cRNA probes. After hybridization, slides were placed against Hyperfilm β-max with ^{14}C-radioactive standards, developed after 6–8 weeks and quantified using computer-assisted densitometry (Quik et al., 1998, 1999).

2.2. Mesencephalic culture model

2.2.1. Cell culture preparation

Mesencephalic cell cultures were prepared as previously described (Jeyarasasingam and Quik, 1999a,b). The ventral mesencephalon from E15 Sprague–Dawley pups was dissected, washed, and mechanically triturated. Following centrifugation at 1000 rpm, the pellet was resuspended and incubated in 0.5% trypsin-ethylenediaminetetraacetic acid. Culture medium (Dulbecco's modified eagle medium supplemented with 18.3 mM glucose, 15 mM HEPES, 0.15% KCl, 1 mM Na_2HPO_4, 10% fetal calf serum and penicillin/streptomycin) was added to stop the reaction. The suspension was centrifuged at 1000 rpm and resuspended in 5-ml culture medium and plated on poly-D-lysine-coated Nunclon 48-well dishes at a density of 3×10^5 cells/cm^2.

2.2.2. Assays

[^{125}I]α-BGT binding to intact mesencephalic cultures and [^{3}H]Epibatidine binding to membranes prepared from mesencephalic cultures were done as previously described (Quik et al., 1997; Davila-Garcia et al., 1999). For tyrosine hydroxylase immunocytochemistry, cultures were fixed in 4% paraformaldehyde, washed in PBS and incubated in PBS with 4% normal goat serum, 1% bovine serum albumin, 1% polyvinyl pyrrolidone, and 0.3% Triton-X-100 for 1 h at room temperature before being incubated in primary tyrosine hydroxylase antibody (Pel-Freez Biologicals) overnight at 4°C. Sections were then rinsed in PBS and bound immunoglobulins visualized using the avidin–biotin immunoperoxidase reaction with the Vectastain ABC-peroxidase kit (Vector, Burlingame, CA). The number of dopaminergic neurons in 15 fields/well was determined by counting cells positively immunostained at a 100× magnification.

3. Results and discussion

3.1. Effect of nigrostriatal degeneration on nicotinic receptor mRNAs in the monkey basal ganglia

3.1.1. Distribution of nicotinic receptor mRNAs in control monkey brain

Nicotinic receptor mRNA localization has been extensively studied in rodent basal ganglia. Rodent substantia nigra contains α3, α4, α5, α6, α7, β2, β3 and β4 subunit mRNAs, whereas localization in the striatum is more limited with low levels of expression of α3, α5, α7 (only in mouse), β2 and β4 mRNA (Dinely-Miller and Patrick, 1992; Marks et al., 1992, 1996; Seguela et al., 1993; Le Novere et al., 1996; Breese et al., 1997; Winzer-Serhan and Leslie, 1997; Charpantier et al., 1998; Elliott et al., 1998). However, in contrast to work in rodent brain, investigations in monkey and human brain are much more limited. Only one study has been done in monkey brain, which reported that α3 nicotinic receptor subunit mRNA was not present in striatum (Cimino et al., 1992). In man, subunit mRNAs, which have been investigated, include α3, α4, α7 and β2 (Court and Clementi, 1995). On the other hand, α4, α7 and β2, but not α3 mRNAs were identified in human striatum (Rubboli et al., 1994; Wevers et al., 1995; Breese et al., 1997), while no data is yet available for the localization of any nicotinic receptor mRNAs in human or monkey substantia nigra.

Because non-human primates represent an excellent model system to study Parkinson's disease (Langston et al., 1999), we investigated the distribution of α4, α6, α7, β2, β3 and β4 mRNAs in squirrel monkey basal ganglia as an approach to subsequently identify alterations in nicotinic receptor subtypes after nigrostriatal damage (Quik et al., 1998, 1999). We selected these subunit mRNAs for study because receptors containing the α4 and β2 subunits comprise the greater majority of neuronal nicotinic receptors in the brain, α7 is the primary subunit of the α-

bungarotoxin nicotinic receptor population, while α6 and β3 subunit mRNAs exhibit a very unique and restricted distribution in the rodent, including their presence in substantia nigra. Although our data show that the distribution of nicotinic receptor mRNAs shared many similarities between monkey and rodent basal ganglia, there were also a number of discrepancies in transcript expression, particularly with respect to expression in the caudate and putamen (Quik et al., 1998, 1999).

Our studies show that α4 and α7 nicotinic receptor mRNAs are present in the caudate and putamen in the monkey (Quik et al., 1998, 1999), a finding which corresponds well to data in human brain (Rubboli et al., 1994; Breese et al., 1997; Gotti et al., 1997; Agulhon et al., 1998; Tohgi et al., 1998). In contrast, however, α4 and α7 subunit mRNAs have not been detected in the striatum of the rat, although α7 but not α4 does appear to be present in the mouse (Wada et al., 1989; Marks et al., 1992, 1996; Seguela et al., 1993; Zoli et al., 1995). These results indicate that there are marked species differences between primates and rodents, which may relate to their very different neuroanatomy, with primates having a separate caudate and putamen while rodents do not. In contrast to the distinct distribution patterns of α4 and α7 in monkeys and rodents, the pattern of expression of α6 mRNA was very similar in the two species, with no detectable labeling in either the caudate or putamen (Quik et al., 1998, 1999). Similarly, expression of the β subunit mRNAs in the monkey striatum resembled that in the rodent, although there were differences in the levels of expression in the two species. A weak β2 and strong β4 nicotinic receptor subunit mRNA signal was observed in the monkey caudate and putamen (Quik et al., 1998, 1999), while no expression of β3 was obtained, consistent with results in the rodent.

In contrast to these discrepant observations between monkeys and rodents in the striatum, there appears to be a relatively good correlation in the distribution of the different nicotinic receptor mRNAs in substantia nigra, although there may be differences in expression levels. Subunit mRNAs identified in monkey substantia nigra in our studies include α4, α6, α7, β2, β3 and β4 (Quik et al., 1998, 1999). These results are similar to those obtained in the rodent substantia nigra, in which α4, α6, β2 and β3 mRNAs have been identified using in situ hybridization and/or RT-PCR (Wada et al., 1989; Marks et al., 1992; Zoli et al., 1995; Le Novere et al., 1996; Winzer-Serhan and Leslie, 1997; Charpantier et al., 1998; Elliott et al., 1998). In situ hybridization and RT-PCR have also localized the β4 transcript to rat substantia nigra, but only in some studies (Winzer-Serhan and Leslie, 1997; Charpantier et al., 1998), while the α7 transcript was only identified in the rodent substantia nigra using RT-PCR (Charpantier et al., 1998; Elliott et al., 1998), presumably because of very low transcript levels. The nicotinic receptor subunit mRNAs remain to be identified in human substantia nigra.

In summary, our results show that α4, α6, α7, β2, β3 and β4 transcripts are present in monkey substantia nigra, while α4, α7, β2 and β4 are localized to the caudate and putamen. Furthermore, they demonstrate that in the substantia nigra, the transcript distribution is similar in rodent and monkey, although the level of expression in this region varied in the two species. On the other hand, there appear to be significant differences in mRNA expression in monkey and rodent striatum, providing further support for primate studies. A knowledge of these species differences is important for the development of appropriate animal models in the design of therapeutic strategies for Parkinson's disease.

3.1.2. Effect of MPTP on nicotinic receptor mRNA distribution in monkey substantia nigra

In our next series of experiments, we investigated the effect of nigrostriatal lesions on nicotinic receptor transcripts in monkey substantia nigra. To date, very few studies have investigated the effect of nigrostriatal damage on nicotinic receptors or their mRNA in the basal ganglia. Clarke and Pert (1985) showed that unilateral 6-hydroxydopamine lesions in the medial forebrain bundle decreased [^{3}H]nicotine binding in the ipsilateral substantia nigra and striatum, while studies in man show that there is an about 50–70% decline in [^{3}H]nicotine binding in the striatum and substantia nigra (Aubert et al., 1992; Perry et al., 1995). Because of the lack of subtype specific ligands, the receptors affected after nigrostriatal damage are not known, although recent studies in rodents have shown that there are declines in α5, α6, β3 (Charpantier et al., 1998; Elliott et al., 1998) and also in α3 and α4 mRNA (Charpantier et al., 1998) using RT-PCR.

We investigated the effect of nigrostriatal degeneration in squirrel monkeys (*S. sciureus*) rendered parkinsonian by systemic administration of the selective dopaminergic neurotoxin MPTP (Quik et al., 1999). One dose of 2 mg/kg MPTP administered subcutaneously resulted in a greater than 50% decline in spontaneous motor activity as compared to saline-treated controls when measured 3–4 weeks after lesioning. The levels of dopamine and HVA in the caudate and putamen were reduced by approximately 50% compared to control animals, and the number of dopaminergic neurons in the substantia nigra was also significantly decreased.

With this moderate lesion, which may reflect the early stages of Parkinson's disease, there were no changes in α4, α7, β2 and β4 mRNA levels in the substantia nigra. Interestingly, α6 mRNA levels were significantly increased and β3 mRNA levels decreased in the substantia nigra after MPTP. Although these data initially appear discrepant from those in the rodent, this most likely relates to the much greater degree of lesioning of the nigrostriatal pathway in the rodent (80–95%) as compared to the monkey (~50%). Studies are currently underway to deter-

mine the effect of a more severe nigrostriatal lesion on nicotinic receptor mRNAs in the monkey.

To conclude, our current data with a moderate nigrostriatal lesion show that there are selective changes in nigral nicotinic receptor subunit mRNA levels in response to nigrostriatal degeneration. These data may suggest that nicotinic receptors containing the α6 and β3 subunits are selectively altered in Parkinson's disease.

3.2. Neuroprotective effect of nicotine in mesencephalic cultures

As mentioned in the Introduction, nicotine administration may attenuate nigrostriatal damage in animal models of neurodegeneration (Carr and Rowell, 1990; Shahi et al., 1991; Janson et al., 1992), while cigarette smoking is inversely correlated with Parkinson's disease (Baron, 1986, 1996; Morens et al., 1995; Balfour and Fagerstrom, 1996; Riggs, 1996; Hellenbrand et al., 1997). In addition, nicotine is protective in several cell culture models of neurotoxicity. For example, nicotine pretreatment prevents glutamate-induced neurotoxicity in striatal, cortical, cerebellar and mesencephalic neurons (Akaike et al., 1994; Marin et al., 1994; Shimohama et al., 1996; Kaneko et al., 1997; Zamani et al., 1997; Maggio et al., 1998; Minana et al., 1998). This nicotine mediated neuroprotection against glutamate toxicity may occur through α7 nicotinic receptor activation (Shimohama et al., 1996; Kaneko et al., 1997). Stimulation of α7 receptors also appears to be neuroprotective against the adverse effects of nerve growth factor deprivation (Meyer et al., 1998) and ethanol-induced cytotoxicity (Li et al., 1999). In addition, nicotine exposure attenuates β-amyloid-induced toxicity, possibly through the activation of α4β2-containing nicotinic receptors (Kihara et al., 1997). The effect of nicotine on MPP^+-induced neurotoxicity, however, has not been investigated.

Because of our interest in the mechanisms of nicotine-mediated neuroprotection against nigral neuron degeneration, and because culture systems have the advantage that they provide a well defined system, which readily allows for mechanistic investigations, we examined the effect of nicotine on MPP^+ toxicity to dopaminergic neurons in vitro. Receptor binding studies were first done to determine whether nicotinic receptors are present on mesencephalic neurons in culture. Robust binding of [^{3}H]epibatidine (2.2 ± 0.4 fmol/10^6 cells), which interacts with α2–α6-containing receptors, was observed as was binding of the α7 receptor selective ligand [^{125}I]α-bungarotoxin (0.5 ± 0.1 fmol/10^6 cells) (Jeyarasasingam and Quik, 1999a). To test for a potential neuroprotective role of nicotine, mesencephalic cells were incubated with 10 μM nicotine for 24 h prior to MPP^+ exposure (3 μM). Such treatment significantly prevented the MPP^+-induced decline in the number of dopaminergic cells by approximately 20% (Jeyarasasingam and Quik, 1999a,b) an effect which was completely blocked by pre-incubation with the nicotinic receptor antagonist d-tubocurarine (Jeyarasasingam and Quik, 1999a). Identification of the nicotinic receptor subtypes, which mediate this effect, is currently under investigation. Both α7- and α4β2-containing nicotinic receptors represent potential candidates since they mediate neuroprotective effects of nicotine in several cell culture models. In view of the restricted localization of the α6 and β3 nicotinic receptor mRNAs to the substantia nigra, a potential role for receptors containing these subunits merits investigation. Furthermore, receptors containing these latter two subunits would provide a highly selective target for agonist therapies.

In summary, our present results demonstrate that nicotinic receptor activation protects nigral dopaminergic neurons from MPP^+ toxicity in vitro. These data suggest that nicotinic agonist therapy may not only improve cognitive and locomotor deficits but also be neuroprotective against the ongoing degenerative process observed in Parkinson's disease.

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