The characterization and subsequent availability of synthetic substance P has renewed interest in the role of this undecapeptide in central-nervous-system function (Mroz & Leeman, 1974). Biochemical and electrophysiological studies have demonstrated that substance P may function as an excitatory transmitter in primary sensory neurons terminating in the spinal cord (Otsuka & Konishi, 1975). In the brain, however, the available evidence is more difficult to interpret.

Using a sensitive radioimmunoassay (Kanazawa & Jessell, 1976), we have previously studied the regional distribution of substance P in the rat brain. Particularly high concentrations of substance P were found in the substantia nigra and interpeduncular nucleus. The interpeduncular nucleus receives afferents from the habenula nuclei (Lenn, 1976), which appear to be primarily cholinergic (Kataska et al., 1973). Substance-P-containing cell bodies have been demonstrated immunohistochemically in the habenula nucleus (Hökfelt et al., 1975) and, in addition, lesions of the habenula appear to decrease substance-P concentrations in the interpeduncular nucleus (Mroz et al., 1976).

The origin of substance P in the substantia nigra is less clear. The substantia nigra receives a major afferent input from the globus pallidum and corpus striatum (Grofova & Rinvik, 1970) and this pathway is thought to contain γ-aminobutyrate (Fonnum et al., 1974). Partial hemisection at the level of the mammillary bodies depletes nigral substance P, suggesting a substance-P pathway originating in either the caudate nucleus or overlying cerebral cortex and passing through the internal capsule (Mroz et al., 1976).

The present study was performed to determine more precisely the origin of substance P fibres projecting to the substantia nigra and interpeduncular nucleus. Stereotactic lesions were placed unilaterally in either the corpus striatum or globus pallidum of male 300g Wistar rats. Lesions were made by using a Radionics radiofrequency lesion generator with a tip temperature of 55–56°C. A second series of rats received lesions ablatting the medial and lateral habenula bilaterally. Then 7 days after lesioning, the rats were decapitated, the brains removed and the brain stems sliced into 300μm coronal sections with a McIlwain tissue chopper in a cold-room at 4°C. The extent of the lesion was verified histologically after sectioning the brain. Nigral or interpeduncular tissue from two consecutive sections was transferred, as separate samples, to small glass tubes for measurement of substance P, glutamate decarboxylase (EC 4.1.1.15) or choline acetyltransferase (EC 2.3.1.6). Substance P was measured by the extraction procedure and radioimmunoassay described previously (Kanazawa & Jessell, 1976). Glutamate decarboxylase and choline acetyltransferase were assayed essentially as described by Fonnum et al. (1970).

After habenula lesions there was a large fall in both the concentration of substance P and choline acetyltransferase activity in the interpeduncular nucleus (see Table 1). A loss of up to 90% of the control choline acetyltransferase activity was found in the interpeduncular nucleus. The decrease in the concentration of substance P paralleled the fall in choline acetyltransferase activity, but was less marked. Approx. 40% of control concentrations of substance P remained after lesions removing 90% of choline acetyltransferase activity. This indicates that there may be a population of substance-P-containing cells or fibres in the interpeduncular nucleus not directly connected to the habenula nuclei. Parallel immunohistochemical studies also demonstrated a marked loss of substance-P-like immunofluorescence in the interpeduncular nucleus after habenula lesions. It appears, therefore, that the habenula–interpeduncular tract is not solely cholinergic but includes a substance-P pathway also.

Biochemical determination of substance P after large pallidal or striatal lesions (see Table 1) suggests that substance-P terminals in the substantia nigra originate in these
Table 1. Effects of lesions on substance-P content and transmitter-synthesizing enzyme activity in the interpeduncular nucleus and substantia nigra of the rat brain

Values quoted are means ± S.E.M. Values in parentheses are the percentage decrease of substance-P content or enzyme activity compared with that in the same nucleus from control animals. N.D., Not determined. *P < 0.01, **P < 0.005, ***P < 0.001, by the Student's t test.

<table>
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<tr>
<th>Region</th>
<th>Substantia nigra</th>
<th>Interpeduncular nucleus</th>
<th>Interpeduncular nucleus</th>
<th>Habenula nuclei</th>
<th>Site of lesion</th>
<th>No. of determinations</th>
<th>Substance P content (ng/g)</th>
<th>Choline acetyltransferase activity (μmol/h per g)</th>
<th>Glutamate decarboxylase activity (μmol/h per g)</th>
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<td></td>
<td>Substantia nigra</td>
<td>Interpeduncular nucleus</td>
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<td>433 ± 93**</td>
<td>186 ± 10**</td>
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<td></td>
<td>1041 ± 159</td>
<td>43.5 ± 9.3**</td>
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<td>43.3 ± 9.3**</td>
<td>1157 ± 85</td>
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<td>441 ± 187</td>
<td>195 ± 20</td>
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<td></td>
<td>164 ± 29**</td>
<td>161 ± 20</td>
<td>N.D.</td>
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</table>

Substantia nigra

Ipsilateral striatum

Ipsilateral pallidum

1977
The magnitude of the fall in substance-P concentration and glutamate decarboxylase activity was well correlated to the size of the striatal or pallidal lesion. Our immunohistochemical studies have shown that substance-P-positive cell bodies are present in the corpus striatum and globus pallidum of the rat. The combination of biochemical immunohistochemical data presented here suggests that substance P in the substantia nigra is localized in the nerve terminals of neurons originating in the globus pallidum and corpus striatum.

P. C. E. is a Beit Memorial Fellow, I. K. is a Wellcome Trust Fellow and T. M. J. is an M.R.C. scholar.

Lenn, N. J. (1976) J. Comp. Neurol. 166, 73–100

Occupancy of Muscarinic Acetylcholine Receptors Stimulates a Guanylate Cyclase in Neuroblastoma Cells

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The muscarinic acetylcholine receptor is known to be involved in the stimulation of smooth-muscle contraction and secretion, and also in the modulation of nerve-impulse transmission in the superior cervical ganglion (Bowman et al., 1971). Ligand binding to the receptor probably leads to a 'cascade' of events inside a cell producing the ultimate tissue response: the 'cascade' may include increased phospholipid turnover, Ca2+ influx, guanylate cyclase stimulation, adenylate cyclase antagonism, or membrane-protein phosphorylation (see, e.g., Michell, 1975).

The binding of agonists and antagonists to the receptor has been extensively studied in brain synaptosomal fractions and ileum (Snyder et al., 1975; Birdsall & Hulme, 1976) showing some correlation with pharmacological data. A study of the 'cascade' of events following receptor activation in whole tissue slices or homogenates is complicated by cellular heterogeneity; also, cell rupture may alter the enzyme activities involved by releasing endogenous neurotransmitters and activators. However, some clones of neuroblastoma cells possess muscarinic acetylcholine receptors (Amano et al., 1972; Matsuzawa & Nirenberg, 1975) and these cells provide a good system for studying the results of receptor activation. They are relatively easy to grow in tissue culture, afford a homogeneous system and have many of the properties of normal nerve cells. In this work clone NIE 115 of the C1300 tumour (Amano et al., 1972) was used, since it is known to give muscarinic responses (Matsuzawa & Nirenberg, 1975). This report describes a preliminary study of the ligand-binding properties of the receptor and the receptor-stimulated guanylate cyclase of these cells.

The receptor-binding properties have been studied on an NIE 115 membrane preparation by using a centrifugation assay (Terenius, 1974) and the ligand [3-3H]quinuclidinyl benzilate. The binding of the pharmacologically active (−)-isomer of this antagonist fits