

of this tubulin would be used at earlier stages of development to allow the formation of the mitotic spindle and therefore proliferation of the undifferentiated nerve cells. At the beginning of the differentiation phase, specific proteins with polymerising activity would appear in increasing amounts, thereby allowing fast microtubule assembly and intensive neurite growth. Thus, the transition between cell division and cell differentiation might be controlled, at least in part, by an increase in the polymerising activity or by qualitative changes in the factors required for

either pathway or both. Although further work is needed to decide on the requirements for microtubule assembly before nerve cell differentiation, our data support the idea that the appearance of specific factors is responsible for the increase in the rates of microtubule assembly during the critical period of brain development—during (and for) intensive neurite growth.

Received 16 November 1979; accepted 29 January 1980.

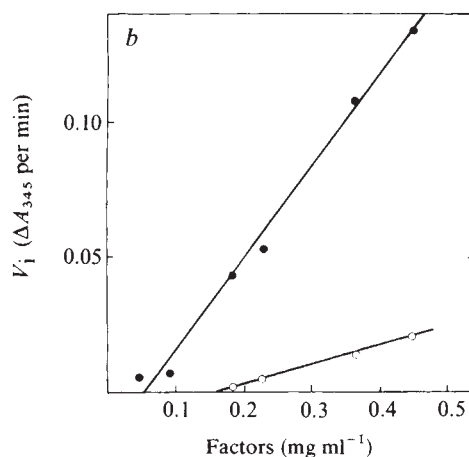
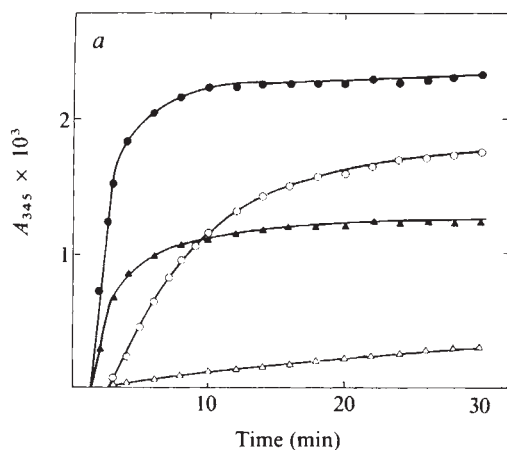


Fig. 3 Polymerisation-promoting activity of adult and 3-d-old rat brain thermostable microtubule-associated proteins on purified tubulin. Purified tubulin was obtained by a procedure previously described by Weingarten *et al.*¹⁰ but slightly modified: activated phosphocellulose was resuspended in the assembly buffer containing (in mM) morpholinoethane sulphonate 100, MgCl₂ 0.5, EGTA 1, EDTA 0.1, GTP 1 and 2-mercaptoethanol 1, pH 6.4. Tubulin was eluted from the column with the same buffer. The purity of the tubulin was controlled by SDS gel electrophoresis. The polymerisation-promoting activity of the two kinds of MAPs on purified tubulin were followed by the turbidimetric method described by Gaskin *et al.*¹⁶. Turbidimetry measurements (345 nm) during tubulin polymerisation, at 37 °C, in the assembly buffer previously described, were carried out using a Carl Zeiss PM 6KS spectrophotometer with an automatic thermostated four-sample changer. *a*, Time course of tubulin polymerisation in the presence of the two preparations of thermostable microtubule-associated proteins. Pure tubulin (0.95 mg ml⁻¹) was incubated in the presence of two concentrations of thermostable MAPs: 'adult' MAPs (▲, 181 µg ml⁻¹; ●, 452 µg ml⁻¹) and '3-d-old' MAPs (△, 181 µg ml⁻¹; ○, 452 µg ml⁻¹). *b*, Relationship between initial rates of tubulin polymerisation and thermostable MAPs concentration. Initial rates of tubulin polymerisation (ΔA₃₄₅ per min) were measured with increasing concentrations of adult MAPs (●) and 3-d-old MAPs (○) and a constant tubulin concentration (0.95 mg ml⁻¹).

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In vivo release of acetylcholinesterase in cat substantia nigra and caudate nucleus

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Acetylcholinesterase (AChE), which is known to inactivate acetylcholine (ACh), is present in great abundance in the substantia nigra, although ACh levels and choline acetylase activity in this region are relatively low¹. Nigral dopaminergic cell bodies and their dendrites also contain AChE^{2–4}. The functional significance of this enzyme in nigro-striatal dopaminergic neurones has been questioned^{1,4,5}. Earlier studies demonstrated an evoked release of AChE from unidentified central neurones into cerebrospinal fluid (CSF) in cats⁶, rabbits^{7,8} and dogs⁹. Later experiments have provided indirect evidence that the substantia nigra may contribute to a substantial amount of AChE detected: a unilateral nigral lesion in rabbits reduced AChE levels in the CSF, whereas electrical stimulation of the substantia nigra induced the opposite effect¹⁰. To investigate the possible release of AChE from dopaminergic dendrites and terminals we measured the *in vivo* release of this enzyme from the substantia nigrae and caudate nuclei of cats implanted with four push-pull cannulae and compared it with that of dopamine (DA). DA is released from dendrites in the substantia nigra^{11,12} as well as nerve terminals in the caudate nucleus. Spontaneous AChE release was observed in the substantia nigra and in the caudate nucleus. Moreover, the application of potassium (30 mM) in one substantia nigra increased the local release of AChE. This was accompanied by remote changes in the enzyme release from the other three structures which differed from that seen for DA. The different patterns of responses observed for AChE and DA suggest that AChE may also originate from other neurones in both the substantia nigra and the caudate nucleus.

Push-pull cannulae were acutely implanted in the two caudate nuclei and substantia nigrae of halothane-anaesthetised cats as described previously¹¹. The four structures were superfused with artificial CSF at a rate of 1.2 ml h⁻¹ and the activity of AChE^{13,14}, nonspecific cholinesterase^{13,14}, and occasionally lactate dehydrogenase¹⁵, were estimated in successive 10-min fractions. In a second series of experiments the release of ³H-DA continuously synthesised from L-[3, 5-³H]-tyrosine (50 Ci mmol⁻¹, 50 μ Ci ml⁻¹) was estimated in the four structures¹⁶. In all cases, the stereotaxic placement of the tip of each push-pull cannula was verified histologically.

A spontaneous release of AChE, which remained constant throughout the experiment, was observed both in the substantia nigrae and in the caudate nuclei. The amount of AChE released was similar in both structures (Fig. 1). Nonspecific cholinesterase was also observed in superfusates, the levels being slightly higher than those of AChE in the substantia nigrae and in the caudate nuclei (Fig. 2).

The addition of potassium chloride (30 mM) for 10 min to the artificial CSF in the left substantia nigra markedly increased the local release of AChE, the maximal change being seen during the treatment. Surprisingly, this effect was accompanied by changes in AChE release in the three other structures. Whereas AChE release was also increased in the contralateral caudate nucleus, it was decreased in the ipsilateral caudate nucleus and the contralateral substantia nigra (Fig. 1). These distal changes were more sustained than those observed in the left substantia

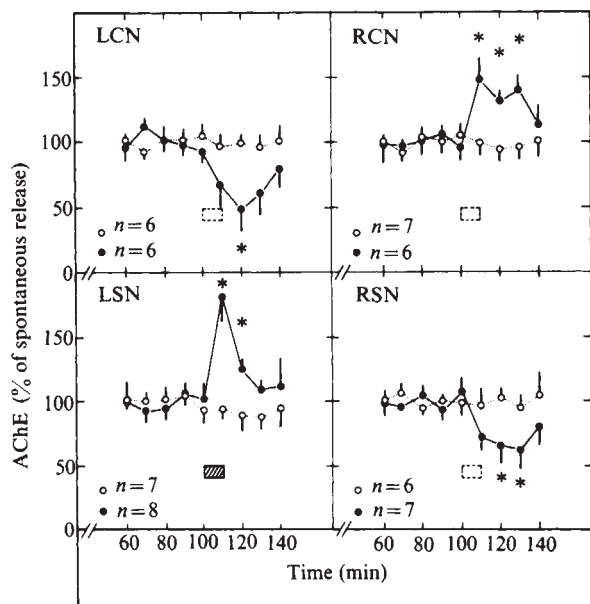


Fig. 1 Effects of the unilateral nigral application of potassium (30 mM) on the release of acetylcholinesterase (AChE) in both substantia nigrae and caudate nuclei. Four push-pull cannulae were simultaneously implanted in the left (LCN) and the right (RCN) caudate nuclei and in the left (LSN) and the right (RSN) substantia nigrae in anaesthetised cats. The four structures were superfused with an artificial CSF (1.2 ml h⁻¹) and AChE was estimated in 10-min successive superfusate fractions 60 min after the beginning of the superfusions. Potassium chloride (30 mM) was applied for 10 min in the LSN (hatched box with solid sides). The empty boxes with dotted sides represent the time during which potassium chloride (30 mM) was applied in the LSN. In each animal, and for each cannula, AChE in each successive fraction was expressed as a percentage of an average spontaneous release calculated from the five fractions collected (1.5 \pm 0.4, 1.8 \pm 0.5 and 1.7 \pm 0.3, 1.6 \pm 0.5 mU ml⁻¹ in the left and right substantia nigra and caudate nucleus respectively). Data are the mean \pm s.e.m. of results obtained with treated animals (●). *P* < 0.05 when compared with corresponding control values obtained in untreated animals (○).

nigra. In contrast, the release of nonspecific cholinesterase was not affected in the left substantia nigra or the other three structures (Fig. 2). As cat plasma contains a substantial amount of nonspecific cholinesterase (269 \pm 47 mU ml⁻¹, *n* = 5) as well as AChE (254 \pm 44 mU ml⁻¹, *n* = 5), the unchanged levels of nonspecific cholinesterase in superfusates would exclude a plasma origin for the observed changes in the release of AChE. Furthermore, the local release of AChE induced by potassium cannot be attributed to gross cell damage as the activity of lactate dehydrogenase (a cytoplasmic enzyme) in the superfusates remained unchanged during potassium application (before, 1.5 \pm 0.6 mU ml⁻¹; after, 1.5 \pm 0.6 mU ml⁻¹; *n* = 4). Therefore, the release of AChE is a specific process.

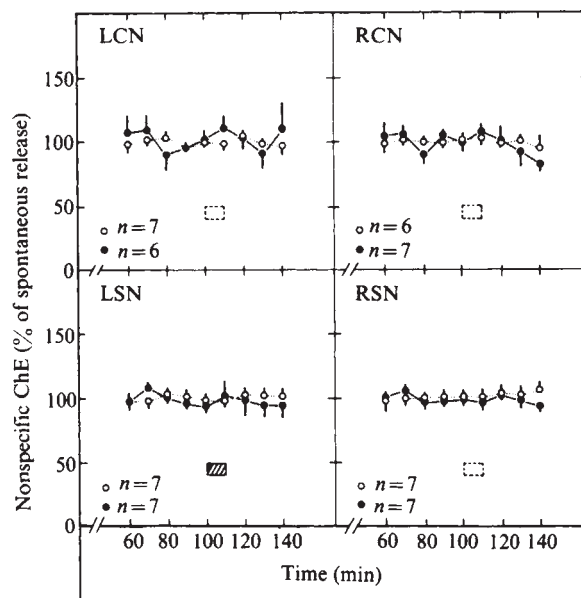


Fig. 2 Lack of effect of the unilateral nigral application of potassium (30 mM) on the release of nonspecific cholinesterase (ChE) in both substantia nigrae and caudate nuclei. During the experiments described in Fig. 1, we also measured nonspecific ChE in 10-min superfusate fractions and expressed the results as described in Fig. 1. The mean spontaneous release of nonspecific ChE in the left and right substantia nigra and in caudate nucleus were 2.3 \pm 0.5, 2.7 \pm 0.7 and 2.4 \pm 0.5, 2.6 \pm 0.6 mU ml⁻¹, respectively. Data are the mean \pm s.e.m. of results obtained with control (○) and treated cats (●). Boxes as in Fig. 1.

Studies on peripheral nerves and innervated tissue have indicated that AChE can be released through a process dependent on nerve activity^{17,18}. The distal changes in AChE release observed in the present study suggest that this phenomenon also occurs in the central nervous system (CNS). There is, therefore, little doubt that the release of AChE in the substantia nigra and caudate nuclei has a physiological significance.

If AChE originates from nigro-striatal dopaminergic neurones, the simplest hypothesis is that AChE should behave as does DA released from dendrites and nerve terminals in the substantia nigra and caudate nucleus respectively. We thus measured the release of newly synthesised ³H-DA in the four structures. Confirming previous results, the unilateral nigral application of potassium (30 mM) enhanced the release of ³H-DA in both caudate nuclei, the effect being slightly less pronounced in the contralateral structure¹⁹. Moreover, potassium increased the release of ³H-DA not only locally in the left substantia nigra but also, surprisingly, in the right substantia nigra. Therefore, the nigral application of potassium induced opposite effects on AChE and ³H-DA release in the ipsilateral

caudate nucleus and the contralateral substantia nigra. These findings do not exclude the possibility of a release of AChE from dopaminergic dendrites and nerve terminals. However, in this case, the messages reaching the ipsilateral caudate nucleus or the contralateral substantia nigra and responsible for the release of AChE and DA would have to be mediated by different pathways, or the two compounds would have to display a differential response to the same signal. Another hypothesis is that AChE originates additionally or exclusively from non-dopaminergic neurones.

Although histochemical and biochemical studies have clearly indicated that a high proportion of AChE is present in dopaminergic cell bodies and their dendrites, nigral AChE also has another location. As shown in the rat substantia nigra, 6-hydroxydopamine lesions, which reduced tyrosine hydroxylase activity by at least 90%, decreased that of AChE by merely 43% (ref. 4). Furthermore, nigral AChE was only reduced by 44% after a local lesion with kainic acid, a neurotoxin which completely destroyed the dopaminergic neurones and is also known to induce the degeneration of other nigral neurones²⁰. AChE not contained in dopaminergic neurones does not originate from striato-nigral fibres, as a kainic acid lesion in the striatum failed to affect nigral levels of AChE²¹. Therefore, afferent fibres from other regions may be involved. The situation is even more complex in the striatum, as in the rat, the dopaminergic terminals only contain about 12% of the entire striatal AChE⁴. Furthermore, kainic acid lesions in the striatum

showed that about 50% of the enzyme was distributed in intrinsic neurones²¹. Therefore, as initially suggested by Shute and Lewis²², various afferent nerve fibres could be rich in AChE. In fact, electrolytic lesions of the centro-median nucleus of the thalamus reduced AChE as well as choline acetyltransferase activities by 50% in the cat²³. Hence, AChE measured in superfusates of the substantiae nigrae and the caudate nuclei could originate from sites other than dopaminergic dendrites or terminals.

Previously, we demonstrated the existence of complex mechanisms involved in the reciprocal control of the activity of the two nigro-striatal dopaminergic pathways. Asymmetric changes in DA release were observed in both caudate nuclei after modifications of DA transmission in one substantia nigra²⁴, or after unilateral sensory stimulation²⁵. These were associated with opposite asymmetric changes in the dendritic release of DA in both substantiae nigrae (reminiscent of the changes in AChE release, Fig. 1). In contrast, symmetric responses in DA release were seen in the two caudate nuclei during modifications of GABAergic or glycinergic transmission in one substantia nigra¹⁹. The existence of two different polysynaptic pathways was postulated to account for the asymmetric and symmetric effects observed¹⁹. Surprisingly, in the same experimental condition, that is, the unilateral nigral application of potassium, two opposite patterns of response for AChE and DA release were seen in the four structures, one being asymmetric (AChE) and the other symmetric (DA). This indicates once more that various neuronal pathways are involved in the reciprocal regulation of the activity of neurones innervating the two substantiae nigrae and caudate nuclei.

Although the origin of AChE released from the substantia nigra and the caudate nucleus has still to be elucidated, these results demonstrate that AChE can be released in the CNS through processes involving nerve activity. It has been suggested that AChE could have a role other than that of inactivating ACh^{1,5}. Before this hypothesis can be verified, it must be established whether or not the distal changes in the release of AChE induced by the unilateral nigral application of potassium are associated with changes in cholinergic transmission. Finally, whatever the mechanisms are in the control of AChE release from both substantiae nigrae and caudate nuclei, they should be taken into account in our subsequent understanding of neuronal interactions within the basal ganglia.

We thank Mr M. Desban for assistance with the histology. This work was supported by grants from INSERM (ATP 71.78.103), DRET (79.077) and Rhône-Poulenc S.G. is a recipient of an INSERM-MRC exchange fellowship, and A.C. is a research fellow of Rhône-Poulenc.

Received 12 December 1979; accepted 4 February 1980.

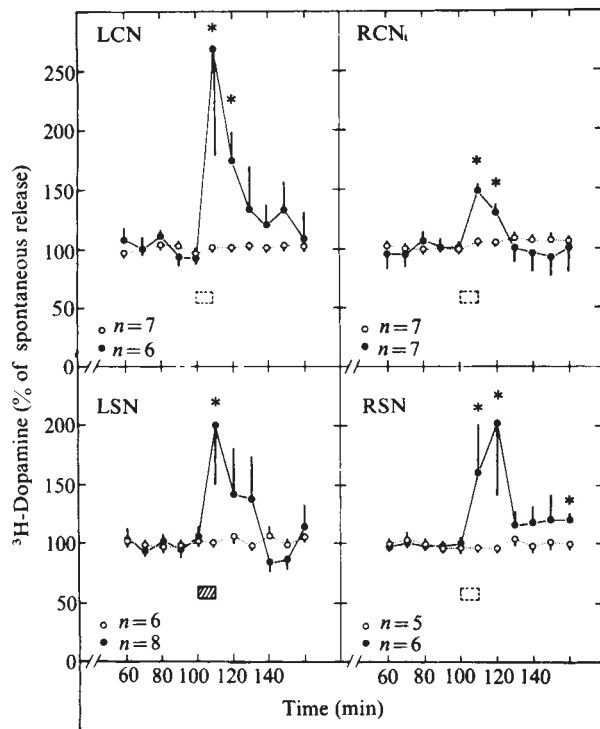


Fig. 3 Effect of the unilateral nigral application of potassium (30 mM) on the release of ³H-DA from the two substantiae nigrae and the two caudate nuclei. The experimental paradigm was essentially the same as described in Fig. 1, except that the artificial CSF delivered to the four push-pull cannulae contained L-[3, 5-³H]tyrosine (50 Ci mmol⁻¹, 50 μ Ci ml⁻¹, 1.2 ml h⁻¹) and that ³H-DA was estimated in 10-min successive superfusate fractions. Results were expressed as described in Fig. 1. The mean spontaneous release of ³H-DA in the left and right substantiae nigrae and left and right caudate nuclei being 0.65 ± 0.07 , 0.57 ± 0.05 and 0.71 ± 0.08 , 0.82 ± 0.10 nCi per 10 min, respectively. Data are the mean \pm s.e.m. of results obtained with treated animals (●), $P < 0.05$ when compared with corresponding control values (○).

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