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A Non-Cholinergic Function for Acetylcholinesterase in the Substantia Nigra: Behavioural Evidence*

S.A. Greenfield, I.W. Chubb**, R.A. Grünewald, Z. Henderson, J. May, S. Portnoy, J. Weston, and M.C. Wright

University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, England

Summary. Acetylcholinesterase is released from substantia nigra neurons, independently of cholinergic transmission. In an attempt to discover the functional significance of this phenomenon, the behavioural effects of injecting acetylcholinesterase into one substantia nigra of the rat were investigated. Following a single injection of the enzyme, intraperitoneal amphetamine evoked circling behaviour in a direction away from the side of injection. Purified acetylcholinesterase with a similar electrophoretic mobility to the endogenous secreted form, was far more potent in eliciting circling than much higher activities of commercial enzyme, consisting of several molecular species of acetylcholinesterase. Similar infusions of butyrylcholinesterase did not induce circling. Depending upon the amount of enzyme initially given, the behavioural effects of a single injection of acetylcholinesterase persisted for up to thirty days. During this period apomorphine, administered systemically, induced transient circling towards the acetylcholinesterase-treated side. It is concluded that secreted acetylcholinesterase has a functional significance within the substantia nigra, independent of cholinergic transmission. This released enzyme could exert long-term changes in the activity of the nigrostriatal system, involving modification of dopamine striatal receptors.

Key words: Acetylcholinesterase – Substantia nigra – Microinfusion – Turning behaviour – Dopamine receptors

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** Permanent address: Department of Physiology, Flinders Medical School, Bedford Park 5042, South Australia

Offprint requests to: S.A. Greenfield (address see above)

Introduction

Acetylcholinesterase (AChE) is released from neurons in the substantia nigra of the cat (Greenfield et al. 1980), rat (Cuello et al. 1981) and rabbit (Greenfield and Smith 1979), almost exclusively from the dopamine (DA)-containing cells of the pars compacta (Greenfield et al. 1983a) and in a calcium-dependent fashion (Greenfield et al. 1983b). Although these neurons appear to be cholinoceptive (Walker et al. 1976; Rotter et al. 1979; Kimura et al. 1981), acetylcholine (ACh) receptors play no part in the release of AChE: release of the enzyme is neither enhanced by cholinomimetics (Cuello et al. 1981) nor prevented by cholinergic receptor blockers (Greenfield and Smith 1979). Furthermore, there is no convincing evidence for cholinergic synapses in the substantia nigra (Lehmann and Fibiger 1978; Dray 1979; Kimura et al. 1981); indeed levels of ACh and choline acetyltransferase are very low (Silver 1974; Levey et al. 1983).

In the substantia nigra, AChE appears more closely related to DA transmission: both compounds are present mainly in the nigro-striatal neurons of the pars compacta (Butcher et al. 1978; Butcher and Woolf 1982) and accumulate in parallel during ontogeny (Butcher and Hodge 1976); both DA (Mercer et al. 1979) and AChE (Liesli et al. 1980) are stored in the smooth endoplasmic reticulum of dendrites; lastly, the release not only of DA (Cheramy et al. 1981) but also AChE (Greenfield and Shaw 1982) is enhanced following application of amphetamine to nigral neurons.

The aim of this study was to see whether or not released AChE has any functional significance in the substantia nigra. It is now well established that rats will exhibit circling behaviour if there is a disparity in the functional amount of DA in the two striata (Pycock 1980) i.e. following a unilateral 6-hydroxy-

dopamine lesion of the nigro-striatal pathway, animals turn in a direction away from the intact side (contraversive rotation) (Ungerstedt 1971). In view of the close relationship between release of AChE and DA, in the substantia nigra, this model was chosen to study the behavioural effects, if any, of infusion of exogenous AChE into one substantia nigra i.e. rats were tested for possible circling behaviour.

Materials and Methods

Animal Preparation

Male Wistar rats (400 g approx.) anaesthetized with chloral hydrate (5% w/v in 0.9% v/v NaCl: 350 mg/kg i.p.) were implanted with guide cannulae (Plastic Products Co.) terminating immediately above one substantia nigra (AP, –7.0; L, 2.0; DV 5.5: skull levelled between bregma and lambda) (König and Klippel 1963). The animals were then left for 2–3 days to allow full recovery from surgery.

Solutions Used for Intracerebral Infusion

AChE: (i) Source, Electric Eel (Sigma); specific activity, 1,400 U/mg protein (1 U hydrolyses 1 μmol of substrate in 1 min).

(ii) Source, foetal bovine serum; specific activity 1,250 U/mg protein. This preparation was highly purified according to the method described by Chubb et al. (1980).

BuChE: Source, horse serum (Sigma); specific activity, 100 U/mg protein.

All enzyme solutions were infused in a volume of 2 μl, at pH7.0. Commercial enzyme preparations were administered in a vehicle of NaCl (0.9% w/v). Purified AChE was administered in a vehicle containing edrophonium (Chubb et al. 1980). All enzyme solutions were assayed on the day of use by a modification (Chubb and Smith 1975a) of the method described by Ellman et al. (1961). Enzymes were infused at the following total activities: – Sigma AChE, 80, 200, 400 and 1,200 mU; BuChE, 2,000 mU; purified AChE, 30 mU.

Polyacrylamide gel electrophoresis (Chubb and Smith 1975a) was performed for Sigma AChE.

Experimental Procedure

(i) Intracerebral infusions: – An inner cannula (Plastic Products Co.) was inserted into the implanted guide cannula such that it protruded 0.5 mm approx. Solutions were infused through the inner cannula connected to a 50 μl Hamilton syringe by capillary tubing (0.025 ins. i.d.) and driven by an automatic pump at a rate of 1 μl/4 min. After 8 min, the pump was switched off but the inner cannula left in place for a subsequent minute. During infusion, the rats were lightly hand-held or freely-moving in a restricted area.

(ii) Tests for circling behaviour: – Before implantation of the outer cannula, all rats were tested for any inherent bias: 20 min following administration of d-amphetamine sulphate (1.5 mg/kg i.p.) they were placed in a circular bowl (12 inch diam.) and the net number of 360° turns/min noted for 30 min. Animals with a mean score of 1 turn/min or more, were not used further. The remaining rats were then implanted with an outer cannula in one substantia nigra (see 'Animal preparation'). After full recovery from surgery,

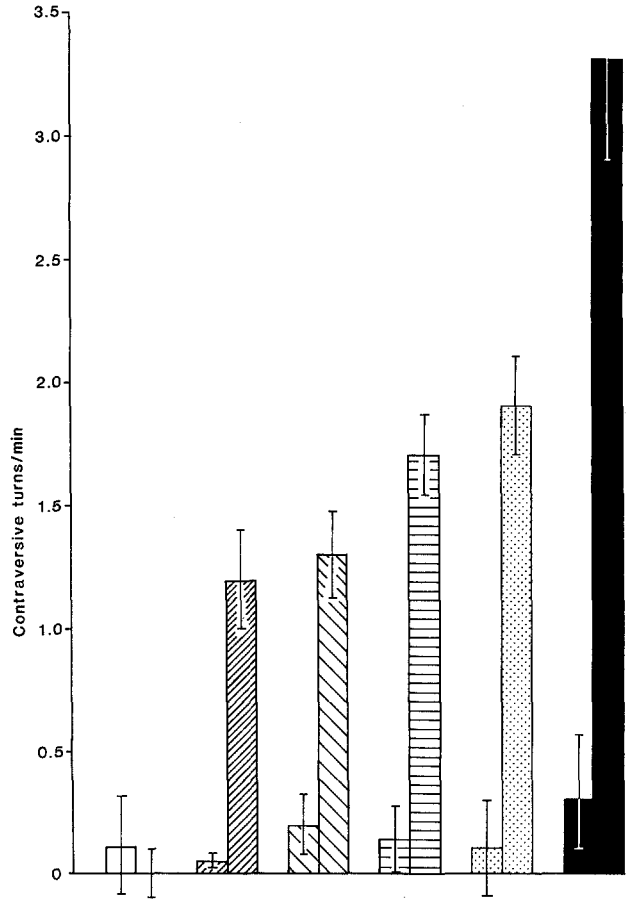

Fig. 1. Net number of 360° turns/minute before and after infusion of different types and amounts of cholinesterase, in six groups of rats treated with amphetamine (1.5 mg/kg i.p.). For each group, the first value shows the mean rate of circling with the outer cannula implanted, but with no infusion: The second value shows the maximum rate of circling following infusion. For all groups except ▨, this maximum level was attained on the same day as cholinesterase treatment, i.e. over the thirty minutes immediately after the infusion was completed. On this day ('1') group ▨ circled at a mean rate of 0.7±0.15 turns/minute in a contraversive direction. The maximum rotation rate for this group (shown) was reached on day 3. Values were calculated, in each group by averaging the mean scores (± S.E.M.) from each animal attained over 30 min.

□ BuChE, 2000 mU/2 μl, n = 5

▨ Commercial AChE, 80 mU/2 μl, n = 5

▨ Commercial AChE, 200 mU/2 μl, n = 10

▨ Commercial AChE, 400 mU/2 μl, n = 9

▨ Commercial AChE, 1200 mU/2 μl, n = 5

■ Purified AChE, 30 mU/2 μl, n = 4

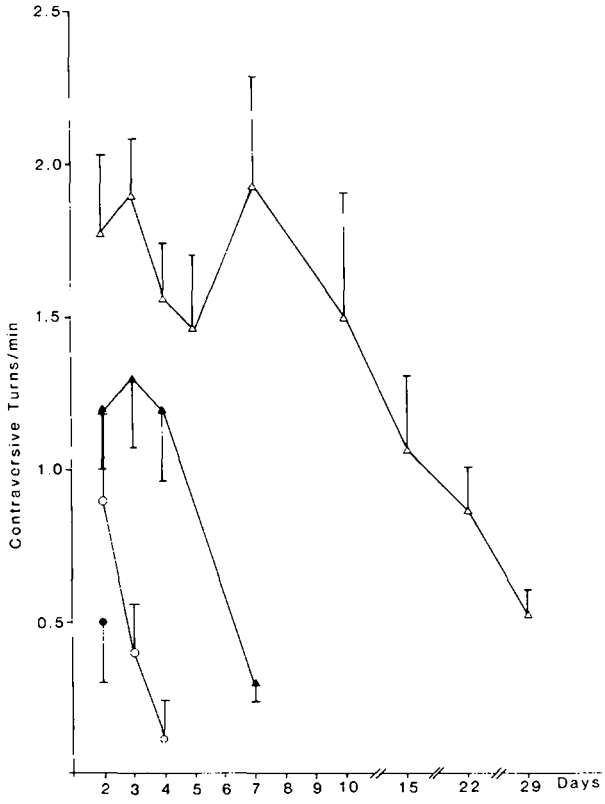

Fig. 2. Long-term effects of a single infusion of AChE, in four groups of rats. Short-term effects of AChE on the same animals ('Day 1') are shown in Fig. 1. 'Day 2' denotes day following infusion when observed animals received no further intracerebral injections, but were challenged with amphetamine (1.5 mg/kg i.p.) and infusion when for rotation. This procedure was repeated on each day indicated. Values show the mean (± S.E.M.) for each group over a thirty minute period, calculated as described in legend to Fig. 1. Each group had received the following amounts of commercial AChE on Day 1:

80 mU/2 µl — n = 5
200 mU/2 µl — n = 10
400 mU/2 µl — n = 9
1200 mU/2 µl — n = 5

the rats were tested for circling as described above, to ascertain whether the implant itself caused a bias in direction of movement. The day prior to infusion of enzyme solutions, most rats received an infusion of 0.9% NaCl (2 µl). Infusions (see (i) above) were performed twenty minutes following administration of amphetamine and rotation observed immediately after the procedure was completed. Enzyme solutions were infused using the same protocol with the rats divided into groups receiving varying amounts and types of cholinesterase (see 'Solutions used for intracerebral infusion'). Each rat received only one treatment of enzyme. On subsequent days, tests for rotation were performed as described above for testing animals prior to surgery.

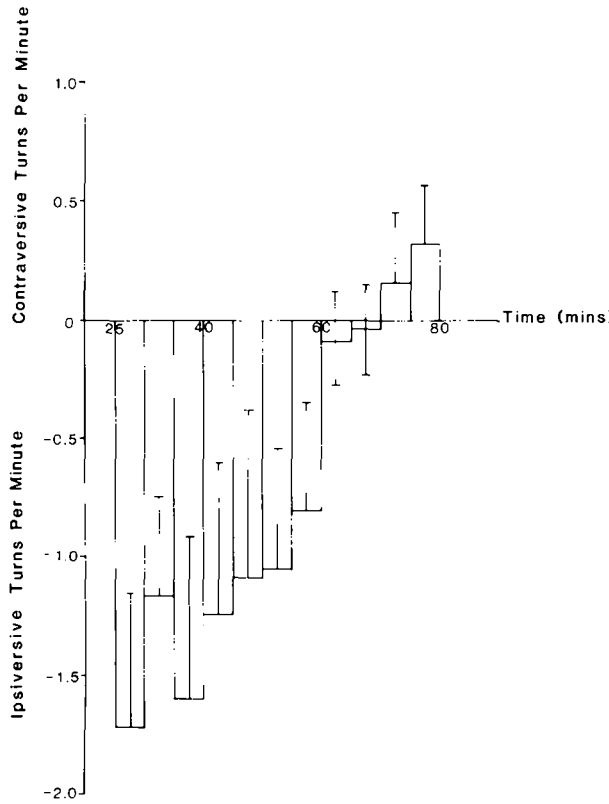

Fig. 3. Effects of administration of apomorphine (1 mg/kg i.p.) during chronic circling behaviour induced by a single infusion of AChE in the presence of amphetamine (1.5 mg/kg i.p.). Nine days earlier the rats (n = 5) had received a nigral infusion of 1,200 mU AChE. Values show the mean rate of circling (± S.E.M.) from the scores of each rat averaged over subsequent five-minute periods, following administration of apomorphine. Note transient change in direction of circling towards the AChE-treated side ('ipsiversive' turning)

Histological Procedures

At the end of the experiment, the animals were killed and the brains removed and stored in 10% (v/v) formal saline at 4° C. Cannulae placements were verified by location on frozen-cut, cresyl-violet stained sections (40 µ). Occasionally, AChE was infused deliberately just above the substantia nigra, as described in the previous section, and the animals killed immediately afterwards. Those brains were then stained for AChE (Lewis 1961), cut in 40 µ sections, and diffusion of the enzyme noted.

Results

Tests for Rotation Before Infusion of AChE

The amount of rotation seen in rats treated with amphetamine before and after implantation of outer cannulae and following infusion of NaCl alone, was negligible. In a total of twenty-two rats the mean turns/minute were as follows: pre-surgery,

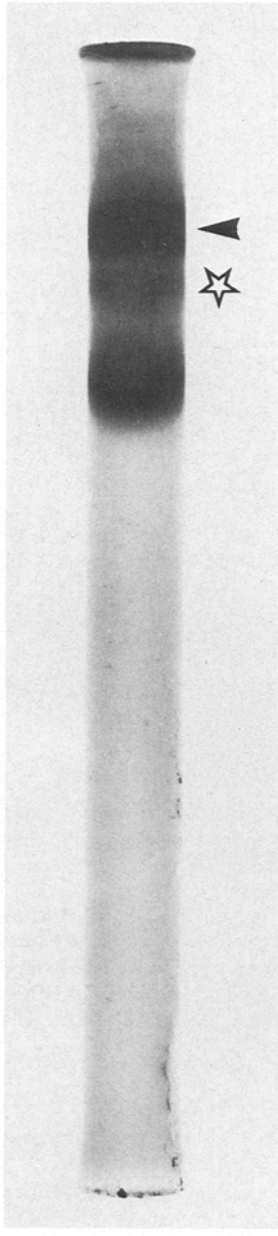

Fig. 4. Polyacrylamide gel electrophoresis demonstrating three isoenzymes in the commercial AChE preparation. The sample was applied at the top of the gel; migration was towards the bottom, the anode. The bands are precipitates of copper thiocholine formed during incubation of the gels, following electrophoresis, according to the procedure described by Chubb and Smith (1975a). The mobility of the heavily-stained band (arrowed) resembles that seen for membrane-bound AChE i.e. it is the slowest: the faint band immediately below (starred) is in a similar relative position, to the membrane-bound form, as the secreted form of AChE (Chubb and Smith 1975a and 1975b)

0.02±0.06; post-surgery, 0.06±0.06; NaCl infusion 0.1±0.04. Infusion of the vehicle of the purified enzyme solution, likewise was without effect.

Effects of Infusion of AChE

AChE administration had no appreciable effect on the motor behaviour of a total of eight rats (0.3±0.3 turns/min, NaCl infusion: 0.35±0.2 turns/min, AChE infusion) in response to amphetamine. These animals were all found subsequently to have cannulae aberrantly implanted outside the substantia nigra (see 'Histological procedures') and were discarded from any further inclusion in analysis. Following infusion of AChE, all remaining rats developed a bias for turning in the direction away from the treated side. There was a tendency for the intensity of circling to be related to the amount of commercial enzyme given (Fig. 1). Purified AChE, at a much lower activity, produced the most vigorous circling (Fig. 1). BuChE given at 2,000 mU, had no effect (Fig. 1).

Long-Term Effects of Infusion of AChE

Contralateral rotation in response to amphetamine following a single infusion of AChE, persisted in a dose-dependent fashion over periods of days (Fig. 2). The group of rats initially given a single infusion of 1,200 mU of AChE into one substantia nigra displayed significant turning behaviour for over 3 weeks (Fig. 2). On the ninth day of this period, these animals were challenged with apomorphine (1 mg/kg i.p.) and subsequently displayed a transient reversal in the direction of circling (Fig. 3). The day previously i.e. eight days following AChE infusion, this group had been circling at 1.8±0.1 (m ± sem) turns/min in a contraversive direction in response to amphetamine. On the day after amorphine treatment, contraversive circling was apparent once more at 1.5±0.2 (m ± sem) turns/min.

Isoenzymes of the Commercial Preparation of AChE

When the commercial AChE preparation was analysed by electrophoresis on polyacrylamide gels, it was found to consist of three detectable isoenzymes (Fig. 4). Of these, the electrophoretic mobility of the most minor component had the closest correspondence to that of the endogenous, secreted enzyme. The electrophoretically slowest isoenzyme was the most intensely stained on the gel, and probably represents membrane-bound AChE, since it is the main constituent of Electric Eel AChE (Massoulie and Bon 1982), and since membrane-bound AChE has a slower electrophoretic mobility than the soluble forms (Chubb and Smith 1975a).

Histological Procedures

Cannulae placements were found to be accurately placed in the substantia nigra of rats that circled following infusion of AChE. A typical section is shown in Fig. 5A. When a section was stained for AChE, the diffusion of exogenous enzyme, deliberately injected just above the substantia nigra, was seen to be not more than 0.5 mm in area (Fig. 5B).

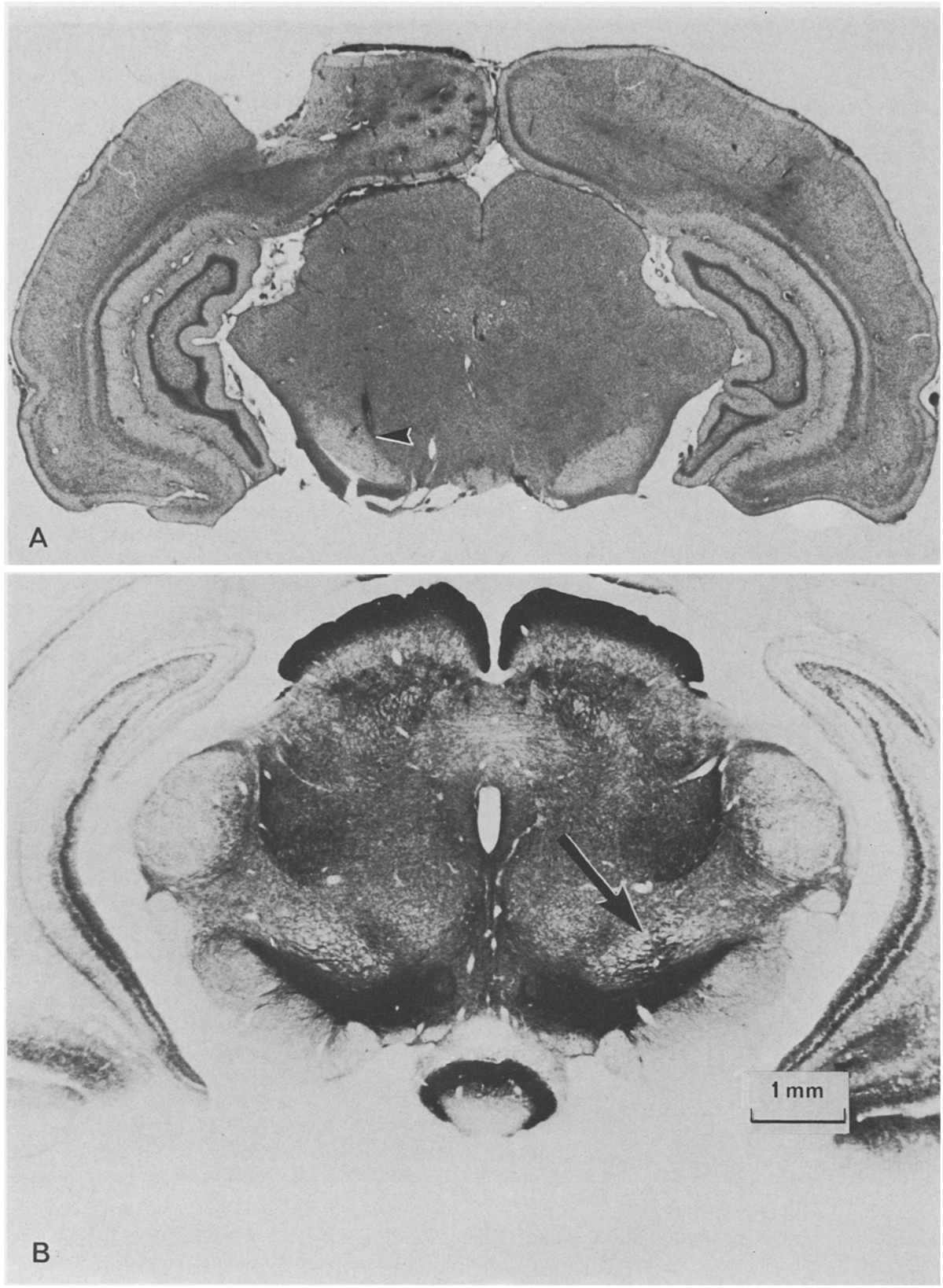

Fig. 5. A Cresyl-violet stained brain section (40 μ) showing placement of cannula (arrowed) just above the substantia nigra. **B** Brain section (40 μ) stained for AChE. In experimental animals with correctly placed cannulae (as in **A**) spread of exogenous AChE was very limited and confined to the AChE containing area of the cannula track and thus difficult to see. To demonstrate AChE spread more effectively an equivalent amount of AChE (200 mU/2 μl) was injected just above the substantia nigra with a fine needle over a period of 8 min at 1 μl/4 min. AChE spread is shown above (arrowed) and is not more than 0.5 mm in area

Discussion

Artefacts that Might Induce Rotation

Neither the presence of the implanted outer cannula nor the infusion of saline alone, produced any significant change in the animals' motor behaviour. Furthermore, the inefficacy of BuChE in inducing rotation indicates that neither infusion of protein per se, nor impurities in the commercial AChE preparation were responsible for the effects observed. In fact impurities in the commercial AChE preparation have previously been shown to be negligible (Greenfield et al. 1981). Furthermore, the potency for eliciting circling, of the purified enzyme, demonstrates that the effects are directly related to AChE itself.

Specificity of Nigral Neurons

It is possible that local application of AChE might have a non-specific effect on any CNS neurons. This explanation for the findings is unlikely: first, rats with aberrantly implanted cannulae did not circle following AChE infusion; second, although the volume injected was relatively large, diffusion of enzyme in the rats that circled, would probably have been restricted to the substantia nigra (Fig. 5B). Indeed, Kreutzberg and Kaija (1974) have shown that AChE diffuses only at about one or two millimetres per hour through the extracellular space.

Is Circling Induced via Hydrolysis of ACh?

Even the lowest activity (80 mU) of commercial AChE used, induced rotation, whereas BuChE given at twenty-five times that amount, did not. BuChE in vitro can hydrolyse ACh at approximately one third the efficiency of AChE (Russell et al. 1974); hence, in the activities given, BuChE would have been as capable as AChE in inactivating endogenous ACh. It would thus appear that the activity of cholinesterase towards the substrate ACh, in the substantia nigra, is not a factor in inducing circling behaviour.

Of the five soluble isoenzymes of AChE (Chubb and Smith 1975a) only one is released from the adrenal medulla (Chubb and Smith 1975b) from the brain into CSF (Greenfield and Smith 1979) and into superfusates of substantia nigra (Greenfield and Shaw 1982). The purified AChE used here consisted solely of this form of the enzyme (Chubb et al. 1980). This preparation of AChE was far more potent in inducing circling than commercial AChE given at far higher activities, but containing considerably less of the secretory isoenzyme. The findings, therefore, suggest: first, the degree of AChE-induced circling is not linked to the amount of activity of the enzyme towards the substrate ACh; second, the rotation observed results from the action on nigral neurons specifically of the single isoenzyme of AChE that is normally released to function in a second, non-cholinergic, capacity.

Is Circling Induced via Hydrolysis of Substance P?

Substance P has been shown, in vitro, to be hydrolysed by the secretory form of AChE (Chubb et al. 1980). Since this peptide is plentiful in the substantia nigra (Cuello et al. 1978) it is conceivable that rotation results as a consequence of its breakdown by the exogenous AChE. Intranigral application of Substance P itself induces contraversive rotation (James and Starr 1977; Olpe and Koella 1977), unless injected into the anterior substantia nigra (James and Starr 1979; Arnt and Scheel-Kruger 1979). The infusions of AChE described in this study were not restricted to anterior substantia nigra and are more comparable to those of Substance P that induced contraversive rotation. In fact, not only behavioural evidence (Pycock 1980) but also biochemical (Cheramy et al. 1981) and electrophysiological (Walker et al. 1976) findings indicate that the overall effect of Substance P in the substantia nigra is to increase release of striatal DA. Nigral application of AChE also leads to contraversive rotation, indicative of an increase in striatal DA release (Dray 1979; Pycock 1980; Ungerstedt 1971) (and see section below). Since nigral application of AChE and Substance P have similar effects, it would be hard to interpret the findings in terms of hydrolysis of the latter by the former. Indeed, Substance P does not evoke release of AChE in the substantia nigra (Cuello et al. 1981) and there is no evidence as yet that, in vivo, endogenous Substance P serves as a substrate for secreted AChE (Greenfield and Shaw 1982).

Long-Term Effects of Micro-Infusion of AChE

The circling induced by AChE persisted, in a dose-dependent manner for up to thirty days following one injection. It is possible but unlikely that these effects could be due directly to the presence of the enzyme remaining in the extracellular space of the substantia nigra. Ungerstedt (1971) demonstrated that following systemic administration of the DA receptor agonist, apomorphine, 6-OHDA lesioned animals circled contraversively away from the side of DA

depletion, due to supersensitivity of striatal DA receptors. Here the ipsiversive circling occurred in the presence of apomorphine, i.e. rats circled towards the side of the nigro-striatal system that had been treated with AChE. Accordingly, this finding suggests that nigral infusion of AChE has led to a sub-sensitivity of striatal DA receptors (cf. Weston and Greenfield 1982) due to a chronic increase in release of striatal DA.

Significance of AChE-induced Rotation

Although contraversive rotation, as seen here following AChE, is associated with an increase in the availability of striatal DA, the neuronal mechanisms involved in this association are not necessarily direct (Pycock 1980).

This point is well-illustrated by the finding that AChE actually depresses the firing rate of pars compacta neurons (Greenfield et al. 1981). Thus, were rotation solely the result of activity of nigro-striatal neurons, ipsiversive turning should have been seen. An analogy, yet not an answer, to this apparent paradox is provided by the actions of γ-aminobutyric acid (GABA) and GABA agonists on nigro-striatal neurons. GABA depresses directly the firing rate of pars compacta cells (Dray 1979; Scarnati and Pacitti 1982) yet causes contraversive rotation (Arnt and Scheel-Kruger 1979; Waddington and Cross 1979; Coward 1982) consistent with a net increase in the activity of nigro-striatal neurons (Oakley and Dray 1978), enhanced release of striatal DA (Cheramy et al. 1978) and possible hyposensitivity of striatal DA receptors (Oakley and Dray 1978). However, this study was not intended to elucidate the complex physiological and biochemical events underlying circling behaviour: rather, it demonstrates that AChE secreted from nigral neurons has a functional significance, independent of cholinergic transmission and possibly not shared by non-secreted forms of the enzyme. The net effect, shown here, of elevating the extracellular nigral concentration of released AChE, is to enhance amphetamine-evoked release of DA in the ipsilateral striatum, for a long period of time. This finding may be relevant to Parkinson's Disease, as it indicates the possible significance of neurosecretory proteins (Greenfield et al. 1983b), in particular AChE, to neuronal homeostasis in the nigro-striatal pathway.

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References

Arnt J, Scheel-Kruger J (1979) GABAergic and glycinergic mechanisms within the substantia nigra: Pharmacological specificity of dopamine independent contralateral turning behaviour and interactions with other neurotransmitters. Psychopharmacology 62: 267–277

Butcher LL, Hodge GK (1976) Postnatal development of acetylcholinesterase in the caudate-putamen nucleus and substantia nigra of rats. Brain Res 106: 223–240

Butcher LL, Talbot K (1978) Acetylcholinesterase in rat nigro-neostriatal neurons: Experimental verification and evidence for cholinergic-dopaminergic interactions in the substantia nigra and caudate-putamen complex. In: Butcher LL (ed) Cholinergic-monoaminergic interactions in the brain. Academic Press, London, pp 25–95

Butcher LL, Woolf NJ (1982) Monoaminergic-cholinergic relationships in the chemical communication matrix of the substantia nigra and neostriatum. Brain Res Bull 9: 475–492

Cheramy A, Nieoullon A, Glowinski J (1978) GABAergic processes involved in the control of dopamine release from nigro-striatal dopaminergic neurons in the cat. Eur J Pharmacol 48: 281–295

Cheramy A, Leviel V, Glowinski J (1981) Dendritic release of dopamine in the substantia nigra. Nature 289: 537–542

Chubb IW, Smith AD (1975a) Isoenzymes of soluble and membrane-bound acetylcholinesterase in bovine splanchnic nerve and adrenal medulla. Proc R Soc B 191: 245–261

Chubb IW, Smith AD (1975b) Release of acetylcholinesterase into the perfusate from the ox adrenal gland. Proc R Soc B 191: 263–269

Chubb IW, Hodgson AJ, White GH (1980) Acetylcholinesterase hydrolyses Substance P. Neuroscience 5: 2065–2072

Coward DM (1982) Nigral action of GABA agonists are enhanced by fluphenazine and differentiated by concommitant flurazepam. Psychopharmacology 76: 294–298

Cuello AC, Emson P, Del Fiacco M, Vale J, Iversen LL, Jessell TM, Kanazawa I, Paxinos G, Quick M (1978) Distribution and release of Substance P in the central nervous system. In: Hughes J (ed) Centrally acting peptides. University Park Press, Baltimore, pp 135–155

Cuello AC, Romero E, Smith AD (1981) In vitro release of acetylcholinesterase from the rat substantia nigra. J Physiol (Lond) 312: 14P

Dray A (1979) The striatum and the substantia nigra: A commentary on their relationships. Neuroscience 4: 1407–1439

Ellman GL, Courtnoy KD, Andres V, Featherstone KM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7: 88–95

Greenfield SA, Smith AD (1979) The influence of electrical stimulation of certain brain regions on the concentration of acetylcholinesterase in rabbit cerebrospinal fluid. Brain Res 177: 445–459

Greenfield SA, Cheramy A, Leviel V, Glowinski J (1980) In vivo release of acetylcholinesterase in the cat substantiae nigrae and caudate nuclei. Nature 284: 355–357

Greenfield SA, Stein JF, Hodgson AJ, Chubb IW (1981) Depression of nigral pars compacta cell discharge by exogenous acetylcholinesterase. Neuroscience 6: 2287–2295

Greenfield SA, Shaw SG (1982) Release of acetylcholinesterase and aminopeptidase in vivo following infusion of amphetamine into the substantia nigra. Neuroscience 7: 2883–2893

Greenfield SA, Grunewald RA, Foley P, Shaw SG (1983a) Origin of various enzymes released from the substantia nigra and caudate nucleus: Effects of 6-hydroxydopamine lesions of the nigro-striatal pathway. J Comp Neurol 214: 87–92

Greenfield SA, Cheramy A, Glowinski J (1983b) Evoked release of proteins from central neurons, in vivo. J Neurochem 40: 1048–1057

James TA, Starr MS (1977) Behavioural and biochemical effects of substance P injected into the substantia nigra of the rat. J Pharm Pharmacol 29: 181–182

James TA, Starr MS (1979) Effects of substance P injected into the substantia nigra. Br J Pharmacol 65: 423–429

Kimura H, McGeer PL, Peng JH, McGeer EG (1981) The central cholinergic system studied by choline acetyltransferase immunohistochemistry in the cat. J Comp Neurol 200: 151–201

König JFR, Klippel RA (1963) In the rat brain: A stereotaxic atlas of the forebrain and lower parts of the brain stem. Williams and Wilkins, Baltimore

Kreutzberg GN, Kaija H (1974) Exogenous acetylcholinesterase as tracer for extracellular pathways in the brain. Histochimie 42: 233–237

Lehmann J, Fibiger HC (1978) Acetylcholinesterase in the substantia nigra and caudate-putamen of the rat: Properties and localization in dopaminergic neurons. J Neurochem 30: 615–624

Levey AI, Wainer BH, Mufson EJ, Mesulam M-M (1983) Co-localization of acetylcholinesterase and choline acetyltransferase in the rat cerebrum. Neuroscience 9: 9–22

Lewis PR (1961) The effect of varying the condition in the Koelle technique. Bibl Anat 2: 11–20

Liesli P, Panula P, Rechardt L (1980) Ultrastructural localization of acetylcholinesterase activity in primary cultures of rat substantia nigra. Histochemistry 70: 7–18

Massoulie JB, Bon S (1982) The molecular forms of cholinesterase and acetylcholinesterase in vertebrates. Ann Rev Neurosci 5: 57–106

Mercer L, Del Fiacco M, Cuello AC (1979) The smooth endoplasmic reticulum as a possible storage site for dendritic dopamine in substantia nigra neurones. Experientia 35: 101–103

Oakley NR, Dray A (1978) The effects of ethanolamine-0-sulphate injection into the rat substantia nigra: Electrophysiological studies. Brain Res 153: 387–391

Olpe H-R, Koella WP (1977) Rotatory behaviour in rats by intranigral application of substance P and an eledoisin fragment. Brain Res 126: 576–579

Pycock CJ (1980) Turning behaviour in animals. Neuroscience 5: 461–514

Rotter A, Birdsall NJM, Field PM, Raisman G (1979) Muscarinic receptors in the central nervous system of the rat II. Distribution of binding of [^{3}H] propylbenzilylcholine mustard in the midbrain and hindbrain. Brain Res Rev 1: 167–183

Russell MA, Jarkan E, Aull JL, Soucie WG (1974) Purification of horse serum cholinesterase by preparative polyacrylamide gel electrophoresis. J Biol Chem 247: 566–571

Scarnati E, Pacitti L (1982) Neuronal responses to iontophoretically applied dopamine, glutamate, and GABA of identified dopaminergic cells in the rat substantia nigra after Kainic-acid induced destruction of the striatum. Exp Brain Res 46: 377–382

Silver A (1974) The biology of the cholinesterases. Elsevier, North Holland, Amsterdam, pp 177–303, 428–431

Ungerstedt U (1971) Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. Acta Phys Scand Suppl 367: 69–93

Waddington JL, Cross AJ (1979) Baclofen and muscimol: Behavioural and neurochemical sequelae of unilateral intranigral administration and effects on ^{3}H-GABA receptor binding. Naunyn Schmiedebergs Arch Pharmacol 306: 275–280

Walker RJ, Kemp JA, Yajima H, Kitagawa K, Woodruff GN (1976) The action of substance P on mesencephalic reticular and substantia nigral neurons of the rat. Experientia 32: 214–215

Weston J, Greenfield SA (1982) Intracerebral microinjections of neuroleptic induces rotation in unlesioned rats. Neuroscience 7 (Supplement): S227

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