can easily be envisaged. This second possibility implies that a change in the phosphorylation state of specific proteins due to a reduction in presynaptic cyclic AMP levels would effectively reduce transmitter release either at the Ca^{2+} influx or at the exocytosis step.

From a series of electrophysiological studies on chick dorsal root ganglion cells in culture, Dunlap and Fishbach have shown that several neuromodulatory agents, including baclofen, noradrenaline, enkephalin, somatostatin and 5-HT, inhibit the calcium-dependent portion of the somatic action potential[17]. This process has been put forward as a model for presynaptic inhibition by these agents. Data from another group suggests that the baclofen-induced inhibition of action potential duration in mammalian dorsal root ganglion cells may be prevented by Cs^+ loading of the cells, implying the involvement of an increased non-resting K^+ conductance[18]. In direct contrast, a voltage-clamp study by Dunlap and Fishbach indicates that a modification of calcium channel conductance is involved[19]. In this study the dibutyryl analogue of cyclic AMP had no effect on the calcium conductance, whereas calcium channels in heart muscle are clearly regulated by cyclic AMP, which mediates the effect of noradrenaline[20].

There are, however, many diverse pieces of evidence suggesting a role for cyclic AMP in enhancing presynaptic efficacy[21,22], although the mechanism(s) involved have yet to be clarified. Activation of most of the other receptors, as well as baclofen, which are negatively coupled to adenylate cyclase has also been shown to inhibit the release of various neurotransmitters. While this circumstantial evidence is striking, further work will be required to determine whether or not inhibition of adenylate cyclase is involved in the inhibitory modulation of neurotransmitter release, or whether it is simply a reflection of another causative process which is common to all these transmitters and occurs in the cell membrane at the same time.

The binding data concerning $GABA_B$ receptors are not sufficiently detailed to answer the question of whether all $GABA_B$ receptors are associated with GTP binding proteins. However, GTP reduced the affinity of baclofen binding to a large proportion of its receptor sites[4]. This does not necessarily indicate that all of these receptors are negatively coupled to adenylate cyclase, as it is beginning to appear that GTP binding proteins are involved in various other receptor-effector mechanisms, apart from positive and negative coupling to adenylate cyclase (for review see Ref. 23). For example, a recent study by Gomperts has shown that Ca^{2+} channel activation in mast cells is GTP-dependent, although whether it involves a new GTP-coupling protein is not yet clear[24]. With respect to the present topic, it will be of great interest to know whether the post-synaptic $GABA_B$-mediated increase in potassium conductance in hippocampal neurones is also dependent on intracellular GTP and whether, if a GTP-binding protein is involved in coupling this receptor to the ion channel which it modulates, it will be the same as that involved in its negative coupling to adenylate cyclase. Answers to this, and the other questions I have outlined in the present review, will require a close partnership between biochemists and electrophysiologists.

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Acetylcholinesterase may have novel functions in the brain

Acetylcholinesterase is generally accepted to be on the surface of cholinergic neurons, where it breaks down the transmitter acetylcholine. But in the brain, acetylcholinesterase distribution is not restricted to cholinergic systems nor is the enzyme always membrane-bound. It can exist in a soluble form within neurons and is sometimes released from them. This diversity in distribution, form and cellular localization suggests a diversity of function. Indeed, both inside and outside the neuron, acetylcholinesterase appears to have actions other than the hydrolysis of acetylcholine.

Acetylcholinesterase (AChE) is an unreliable marker for cholinergic transmission. Awareness of this has grown, over the last twenty years, as a result of studies of AChE on non-neuronal tissue as well as in the developing and adult nervous systems[1]. I shall concentrate mainly on neurons in the adult brain and examine the evidence suggesting that the presence of AChE is not necessarily indicative of cerebral cholinergic systems. Nevertheless, it is possible that any considerations arising can also be extended to AChE-containing cells outside the brain. The most critical issue then is to discuss whether 'non-cholinergic' AChE represents a caprice of nature or underlies hitherto unknown neuronal mechanisms.

Disparity in the distribution of AChE and choline acetyltransferase

Choline acetyltransferase (ChAT) is the synthesizing enzyme for acetylcholine and is normally regarded as the most faithful marker of the cholinergic neuron. However, in certain brain regions AChE is present in abundance whilst ChAT activity is very low. In the first key review on this subject[1] the regions cited were: substantia nigra, cerebellum, globus

pallidus and hypothalamus. The disparity in distribution of the cholinergic enzymes was originally demonstrated by comparing the density of histochemical reaction for AChE with the ChAT content of homogenates of the same area.

Indeed, just comparisons of the AChE histochemical distribution in different species, could raise doubts as to the accuracy of the enzyme as a cholinergic marker. In the guinea-pig cerebellum, there is twice as much AChE in the molecular layer as in the granular layer, while the opposite is true of the rabbit and cat. Hence, as Karczmar[2] pointed out, it would be strange if cholinergic synapses are present in the molecular layer of the guinea-pig but not in that of the related species, the rabbit. Apart from this type of observation, however, comparisons of the histochemical distribution of AChE with the biochemical distribution of ChAT can only be qualitative.

A more quantitative means of assessing that AChE is in excess of cholinergic requirements is to assay both ChAT and AChE in the same homogenates of a particular brain region[1]. For example, the cerebellar cortex has only 2.2% of the ChAT activity of the caudate nucleus but 26.7% of the AChE activity[3]. However, we could question why the caudate nucleus should be taken as this type of yardstick. A 'normal' ratio of ChAT to AChE might not exist, but rather depend on how efficiently acetylcholine needs to be removed in specific neuronal networks. Disparity in distribution of ChAT and AChE should therefore be viewed with caution. It is suggestive, but not a direct demonstration of, a possible association of AChE with non-cholinergic systems.

Due to the advent of ChAT immunocytochemistry such demonstrations are now within reach – it has been possible to compare AChE and ChAT at the single neuron level. Studies[4] using this technique support the interpretation of earlier work[1] (and above) in that they show groups of individual neurons containing AChE but devoid of ChAT (Fig. 1). Furthermore, even in a brain structure as familiarly 'cholinergic' as the caudate nucleus, it has now been shown that subpopulations of cells contain only AChE (Ref. 5).

It is possible that AChE-containing cells, whatever their transmitter, receive a cholinergic input: the enzyme would then be present post-synaptically to hydrolyse acetylcholine. However, the following three examples illustrate that such conditions must be actively demonstrated rather than simply assumed:

(1) AChE is not necessarily present in the target cell of a cholinergic synapse. In the striatum, over 90% of the cells ('medium spiny neurons') do not contain AChE, but probably receive a cholinergic input[5,6];

(2) cells can be cholinoceptive (i.e. excited by iontophoretic acetylcholine) without receiving a cholinergic input. Purkinje cells in the cerebellum are cholinoceptive but stimulation via their afferent inputs is not modified by cholinergic agents[7]. Furthermore, even though these cells are cholinoceptive, they do not contain AChE (Ref. 1);

(3) neurons can contain large amounts of AChE and also be cholinoceptive, without an identifiable cholinergic afferent pathway, e.g. nigro-striatal cells[8].

At the moment it is impossible to list all neuronal groups in the brain where AChE exists beyond the requirements, or in the absence of, cholinergic transmission. Nevertheless, neurons where this phenomenon may occur are found in the following areas: cerebellum, substantia nigra, lateral hypothalamus, zona incerta, caudate nucleus, locus coeruleus, globus pallidus and dorsal raphé. Of course the fact that AChE is present, either alone or in excessive amounts, does not prove the enzyme has non-cholinergic functions in the brain: it is however a cogent, though not sufficient, basis for the argument.

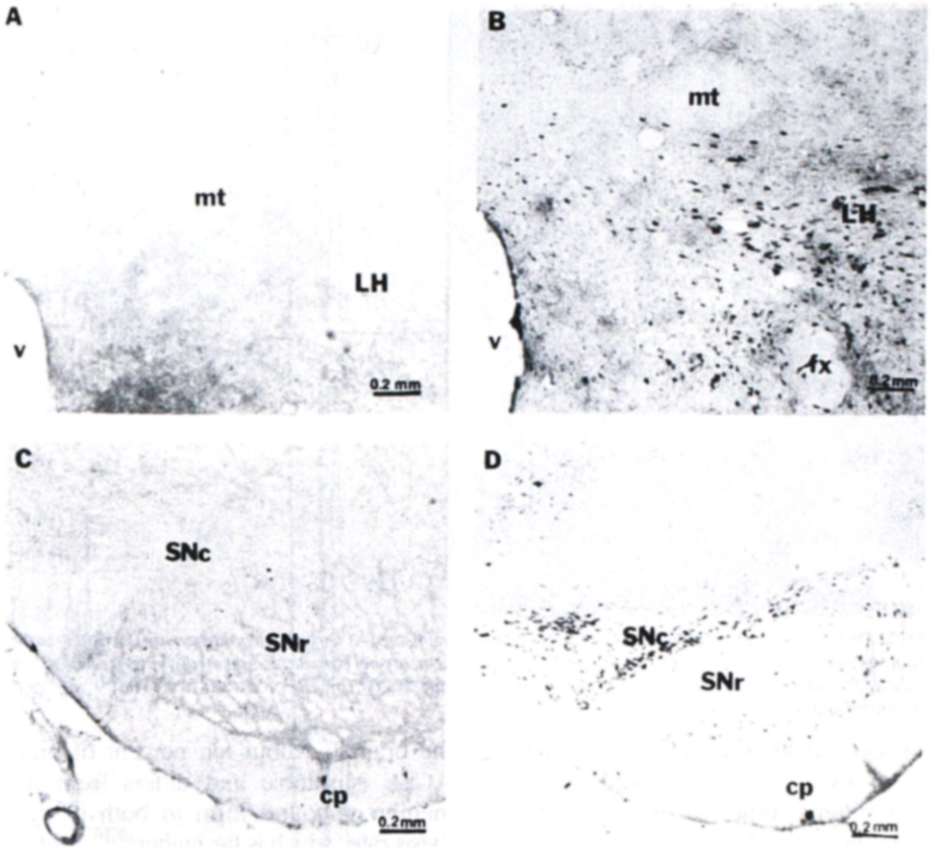

Fig. 1. *Photomicrographs of the lateral hypothalamus and substantia nigra in adjacent tissue sections, stained for either choline acetyltransferase or acetylcholinesterase.*
(A) *and* **(B):** *Low power of lateral hypothalamus stained for either ChAT* **(A)** *or AChE* **(B).**
(C) *and* **(D):** *Low power of substantia nigra stained for either ChAT* **(C)** *or AChE* **(D).** *There were no ChAT positive neurons in either region, while there were abundant AChE-stained neurons in both regions.*
Abbreviations: LH, lateral hypothalamus; SN, substantia nigra, pars compacta (SNc) and pars reticulata (SNr); cp, cerebral peduncle; fx, fornix; mt, mammillothalamic tract. Reproduced from Ref. 4 with permission of authors and Pergamon Press Ltd.

Co-localization of AChE with other transmitters

If AChE is not contained within cholinergic neurons then is it associated with another particular class of transmitters? Answering this question might help us to identify a possible non-cholinergic function for AChE.

Butcher and his colleagues have beautifully demonstrated that AChE is present in noradrenergic neurons in the locus coeruleus[9] (Fig. 2) and also in dopaminergic cells in the substantia nigra[8]. However, it may be rash to generalize that 'non-cholinergic' AChE is always stored with catecholamines. There is indirect evidence to suggest that within the caudate nucleus AChE is present in both somatostatin- and GABA-containing neurons[5]. In addition, AChE is found in close association with Substance P-containing cells in both the retina[10] and the dorsal horn of the spinal cord[11].

We are led then to formulate possible non-cholinergic functions for AChE in neurons devoid of ChAT; perhaps the

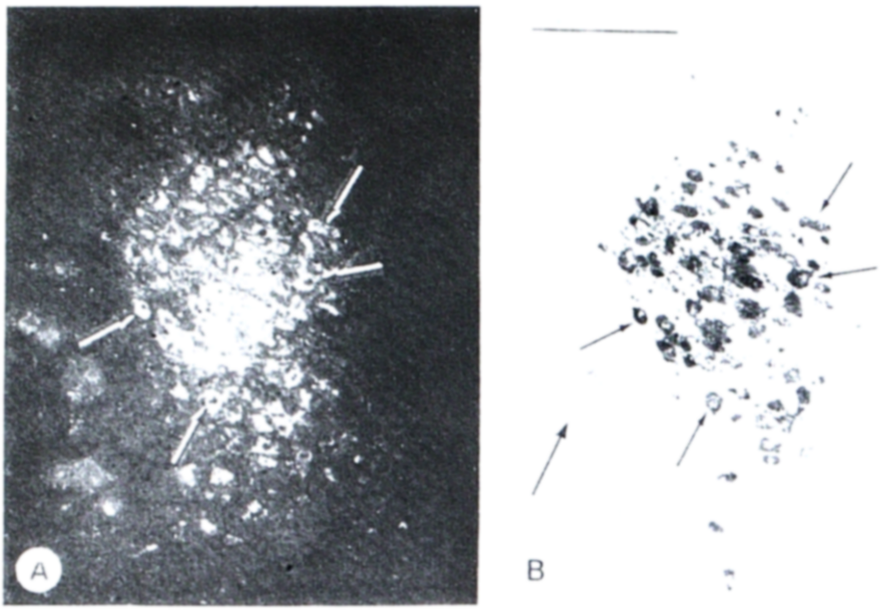

Fig. 2. *Demonstration in rostral locus coeruleus of norepinephrine* **(A)** *and acetylcholinesterase* **(B)** *on the same brain section. Small arrows in* **(A)** *and* **(B)** *point to the same neuronal somata. Large arrow in* **(B)** *points to cell body in the mesencephalic nucleus of cranial nerve V. Scale = 200 μm.* Reproduced from Ref. 9 with permission of authors and Elsevier.

studies cited above are just a few examples of co-storage of AChE with identified transmitters, other than acetylcholine. Even though we do not have an exhaustive inventory of every transmitter in every AChE-containing non-cholinergic cell, we can still see that the enzyme is not limited to association with any one type of transmitter. Clearly then, it is difficult to imagine a role applicable to diverse transmitter systems, irrespective of the physiology of individual neuronal networks. For example, in the caudate nucleus, AChE is distributed in patches, 'striosomes', in register with other striosomes containing opiate peptides[12]. These striosomes, and hence AChE, may have a significance specific to the organization of the striatum alone.

Another important reason for rejecting the idea of a uniform second function is that 'non-cholinergic' AChE can vary in its subcellular localization. In some non-cholinergic cells the enzyme is present in the Golgi apparatus (e.g. dopaminergic nigral neurons[6]) whereas in others it is not (e.g. non-dopaminergic nigral neurons[6], striatal neurons[5]). We know that in cultures of muscle cells, AChE must acquire complex sugars in the Golgi apparatus, prior to externalization[13]. It is possible therefore that non-cholinergic AChE could have at least two functions – one inside the cell and another necessitating association with, or passage through, the plasma membrane, and hence requiring processing in the Golgi apparatus.

Soluble AChE

Despite its well-known position on the surface of cells, AChE also exists in a soluble form, in both the periphery[14] and the brain[15]. About ten percent of brain AChE is soluble and differs from the membrane-bound form in both its synthesis rate, which is far higher[13,16], and its charge, which is more negative[14]. What could be the function of this soluble AChE? As we have seen (in the section on co-localization of AChE with other transmitters), in some non-cholinergic cells the enzyme is not located in the Golgi apparatus. Therefore, AChE may be soluble in these neurons, and have an intracellular role. This role will surely depend on the transmitter synthesized in that cell and we shall explore a specific example in the next section (Non-cholinergic actions of AChE).

Soluble AChE may also have a function in the extracellular space. This idea has arisen from the observation that the enzyme is present in cerebrospinal fluid (CSF) and represents a physiological release process from neurons in the brain[17]. Although there are five soluble isoenzymes of AChE only one, the least negatively charged, is released[17]. This AChE in CSF consists of four catalytic subunits with no collagen tail; this is the G4 form[18]. In certain tissues, AChE is polymorphic, i.e. varies in size and shape; in the brain however, the G4 form is the most common[18].

Interestingly enough, levels of AChE in CSF do not correspond to release of acetylcholine:

(1) brain acetylcholine is released mainly into the lateral ventricle, whereas the highest concentration of AChE is found in the cisterna magna;

(2) release of acetylcholine into the ventricular system is depressed by anaesthesia, but release of AChE is not[19,20];

(3) when certain brain areas (i.e. caudate nucleus, substantia nigra) are stimulated electrically, there is an increase of AChE in cisternal CSF that is not blocked by

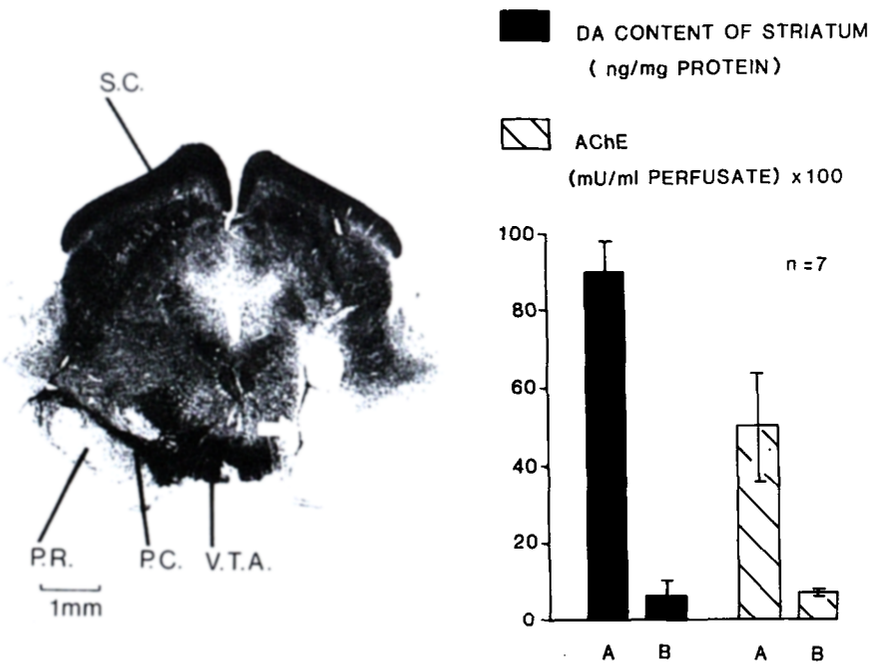

Fig. 3. *Effects of 6-hydroxydopamine lesions of the nigrostriatal pathway on distribution and release of acetylcholinesterase in the substantia nigra. The section of rat brain is stained for AChE. Eight weeks earlier the animal had undergone a unilateral 6-hydroxydopamine lesion of the nigro-striatal pathway on the right-hand side. Note decrease of AChE reaction, on lesioned side, in substantia nigra pars compacta and pars reticulata and ventral tegmental area.*

Arrow indicates tract of push-pull cannula, used for local perfusion. PC, substantia nigra pars compacta; PR, substantia nigra pars reticulata; SC, superior colliculus; VTA, ventral tegmental area.

Histogram shows AChE concentration in perfusates of substantia nigra of control rats (A, n=7) and lesioned rats (B, n=7). There is far less release of AChE in lesioned animals, comparable to the amount of dopamine (DA) depletion in their striata. Modified from Ref. 23 and reproduced with permission of Alan R. Liss, Inc.

cholinergic receptor antagonists[21].

Indeed, much of the AChE in CSF originates from nigrostriatal neurons[21]. Curiously, however, the enzyme is released not only from their terminals in the caudate nucleus, but also from their somata/dendrites within the substantia nigra[22,23] (Fig. 3). Dendritic release of AChE has already been postulated in the guinea-pig facial nucleus, where AChE-reactive smooth endoplasmic reticulum (SER) is sometimes in apposition with the dendritic membrane[24]. AChE is also present in the SER of nigro-striatal cell dendrites. Furthermore, these neurons have abundant rough endoplasmic reticulum, whereas non-dopaminergic cells contain far less[25]. This situation probably indicates that protein such as AChE is being manufactured at a much more rapid rate in nigral dopaminergic neurons[25]. High turnover would surely be a prerequisite for release of AChE, and has indeed been shown to be the case for the enzyme secreted in cultures of muscle[13]. In addition, nigro-striatal cells have an AChE-reactive Golgi apparatus (cf. section on co-localization of AChE with other transmitters) whereas other AChE-containing nigral neurons do not[10]. These anatomical observations support neurochemical studies[23] showing that, although non-dopaminergic nigral neurons contain AChE[8], only dopamine-containing nigro-striatal neurons release the enzyme (see Fig. 3).

So far, the possible release of AChE from other non-cholinergic neuronal populations has not been investigated. Nevertheless it is already apparent that the enzyme can be released in at least two ways: firstly from nerve terminals, as in the caudate nucleus; secondly from dendrites, as in the substantia nigra. It would be erroneous to infer that release of AChE always occurs independent of cholinergic systems (for comparison see Ref. 19), although within the substantia nigra at least, this seems to be the case. There is no ChAT in nigro-striatal neurons and no cholinergic input (see section on disparity in the distribution of AChE and choline-acetyltransferase), furthermore release of AChE from nigro-striatal cell dendrites is not enhanced by cholinomimetics[26], even though the cells are cholinoceptive. The question remains, however, whether soluble AChE has any action beyond the hydrolysis of acetylcholine, either inside or outside the neuron.

Non-cholinergic actions of AChE

One means of investigating novel roles of AChE is to study the effects of exogenous enzyme. So far, this approach

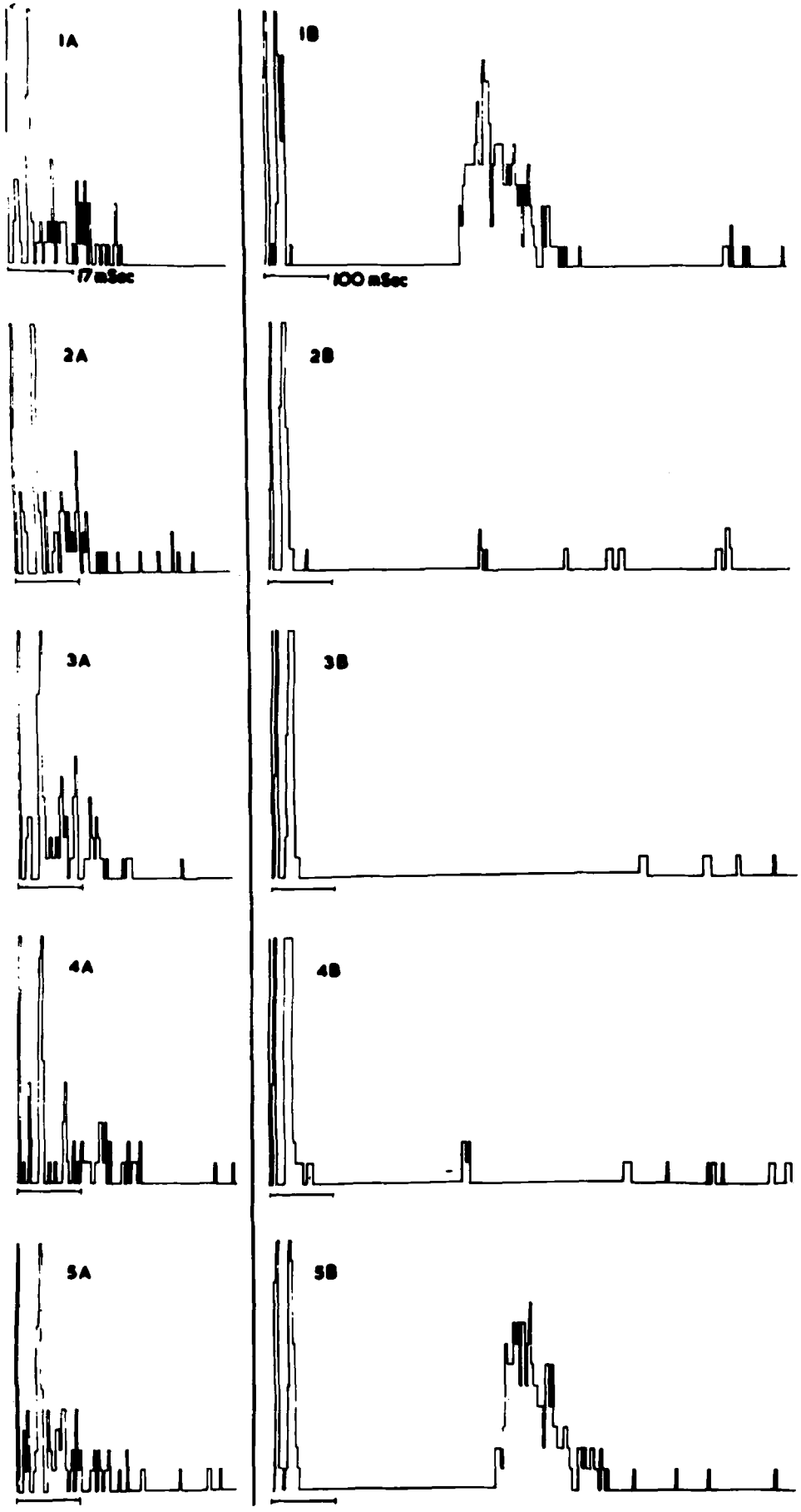

Fig. 4. *Effects of micro-injection of purified acetylcholinesterase on discharge of a slow-firing nigral cell following caudate nucleus stimulation (64 sweeps).* **1A** *and* **1B** *control.* **2A** *and* **2B** *3 min after applying AChE.* **3A** *and* **3B** *6 min after applying AChE.* **4A** *and* **4B** *9 min after applying AChE.* **5A** *and* **5B** *12 min after applying AChE.* **1,2,3,4,5 A** *fast time base to show early excitation.* **1,2,3,4,5 B** *slower time base to show entire response following caudate nucleus stimulation. Exogenous AChE does not affect early excitation, that is probably mediated by Substance P, but prolongs the inhibition which is probably mediated by GABA and abolishes 'rebound' excitation.* Reproduced from Ref. 28 with the permission of Pergamon Press Ltd.

Table I. Reasons for believing AChE could have novel functions in the brain.

In certain brain regions there are large amounts of AChE but very little ChAT.
AChE can be present in neurons that have no ChAT, nor any identified cholinergic input and that employ transmitters other than ACh.
AChE exists not only in its familiar membrane-bound position but also as a soluble endo- and exo-enzyme.
Release of AChE into cerebrospinal fluid does not parallel release of ACh.
Release of AChE is not necessarily affected by stimulation or blockade of the ACh receptor.
AChE has various physiological actions unrelated to the hydrolysis of ACh.

has only been used in the retina and the substantia nigra. Nevertheless, the respective findings illustrate at least two possible, non-cholinergic functions for AChE, one intracellular, the other extracellular.

Chubb and his colleagues have shown that *in vitro*, AChE has carboxy- and amino-peptidase activity towards Substance P and the enkephalins (both met- and leu-enkephalin) but not towards somatostatin, oxytocin, vasopressin, ß-endorphin and bovine serum albumin. Of more relevance to the current argument is the demonstration that the peptidase site is probably not the same, on the AChE molecule, as that for its esterase action[11].

Both this relative substrate-specificity and non-cholinergic action appears to have physiological significance. In amacrine cells in the retina, AChE is present in enkephalin- and Substance P-containing neurons, but not in somatostatin-containing neurons. Following depletion of retinal peptides by light deprivation, incubation with exogenous AChE markedly enhances the immunocytochemical reaction for enkephalin and Substance P. Hence AChE may have an intracellular function in the processing of these peptides[27].

In the substantia nigra, we have simulated the release of AChE by local microinfusion of the enzyme *in vivo*. We found that AChE, but not butyrylcholinesterase, depresses both the spontaneous and stimulus-linked firing of identified nigro-striatal cells[28] (Fig. 4). Exogenous AChE applied to nigral neurons also has effects on motor behaviour that last for relatively long periods of time and seem to involve modification of striatal dopamine receptors. Again, these behavioural effects are unrelated to the activity of AChE towards acetylcholine[29].

It seems highly unlikely that AChE is performing the same non-cholinergic role in the retina and nigro-striatal cells. AChE released from nigro-striatal neurons is not an aminopeptidase that will break down leucine-ß-naphthylamide[30]. Furthermore, neither the electrophysiological[28] (Fig. 4) nor the behavioural effects[29] of exogenous AChE appear to be mediated via breakdown of Substance P. Nevertheless, it is possible that in the non-dopaminergic cells of the substantia nigra, AChE could play an intracellular role more akin to that seen in the retina.

As yet, no attempts have been made to explore the function of AChE released from nigro-striatal cell terminals. All that can be said at the moment is that the release of the enzyme within the striatum appears to reflect the activity of the nigro-striatal pathway[30,31].

By contrast, dendritic release of AChE seems unrelated to frequency of somatic discharge of nigro-striatal neurons[30,31], but may instead be regulated by local dendritic calcium currents[32]. Furthermore, there are no dendro-dendritic synapses in the substantia nigra: dendritically released substances would thus diffuse in a relatively non-restricted manner. Hence, as already postulated for dendritic release of dopamine[33], release of AChE from nigro-striatal neurons may subserve a different form of interneuronal communication from that seen in normal synaptic transmission, i.e. neuromodulation. Both dopamine and AChE could act as modulators by changing the sensitivity of the nigro-striatal cell body to signals received at its distal dendrites[32].

Conclusions

The reasons for believing AChE could have novel functions in the brain, are summarized in Table I. As described in the preceding sections, the evidence hinges on a dissociation of AChE from cholinergic systems, demonstrated with the techniques of neuroanatomy, cell biology, biochemistry, neurophysiology, pharmacology and behaviour. However it is one thing to show AChE is not performing its usual function but quite another to say what the other function, or functions, might be. The problem is made harder by the lack of uniformity emerging from studies of 'non-cholinergic' AChE. In some neurons it is co-localized with catecholamines, in others with peptides. In some non-cholinergic cells it is present in the Golgi apparatus and in others it is not. It is released from some neurons, but not from others. The release itself can occur not only from terminals but also from dendrites, reflecting cell firing in the former case, but not in the latter.

Exactly how AChE performs these non-cholinergic intra- and extra-cellular functions awaits elucidation. Furthermore, until we know more about other non-cholinergic but AChE-containing neuronal populations, we will not know how general these functions are, nor how many others exist.

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