The Action of Acetylcholine on Cells in Cat Cerebellar Cortex

The presence in cerebellar cortex of large amounts of acetylcholinesterase[1] coupled with its low content of choline acetylase[2] and acetylcholine[3] has given rise to considerable speculation about the role of acetylcholine in this portion of the central nervous system. It has frequently been assumed that acetylcholine exerts an excitant action on neurones in the cerebellar cortex and that the function of the cholinesterase is to prevent excessive concentrations of acetylcholine reaching these neurones[4]. Injection of acetylcholine into the internal carotid artery after occlusion of the vertebral arteries provoked a marked increase in cerebellar activity[5], but this may well have been secondary to an action on neurones in the brain stem. More recently, topically applied acetylcholine has been demonstrated to have an excitant action on the cerebellar cortex[6], but this route of administration also suffers from the disadvantages of possible alterations in vascular supply and lack of specificity in localization.

We have used five-barrelled micropipettes to study the responses of 562 cells in the cerebellar cortices of 18 cats. The central barrel contained 4 *M* NaCl and recorded the extracellular activity of single neurones, while the other four contained saturated solutions of the drugs to be tested, which could be released iontophoretically in the immediate vicinity of individual neurones.

In the initial series of experiments cats were anaesthetized with Dial compound (Ciba), and of the 350 cells tested, 9% were found to be sensitive to an excitant action of acetylcholine. In a further series of 6 cats anaesthetized with pentobarbital sodium, we recorded from 150 cells, of which 39% were excited by acetylcholine. We did not locate any cells which were inhibited by acetylcholine in either series of experiments. Administration of Dial compound to one cat in the second series was found to depress severely the responses of cells to acetylcholine, in the absence of any sustained alterations in femoral arterial blood pressure and without altering the sensitivity to iontophoretically applied glutamate ion, passed from another barrel of the pipette. A further total of 62 cells was tested in two cats decerebrated by precollicular transection. Of these 18% were excited by acetylcholine, suggesting that pentobarbital sodium does not depress acetylcholine sensitivity to any marked extent. The results with cats anaesthetized with Dial were somewhat unexpected since it had been used as the standard anaesthetic in a previous survey on Betz cells in the cerebral cortex[7] without any indication of a specific depressant action. Cells were located at depths of 100 μ to 4 mm (which was the greatest depth explored). Typically, those that responded to acetylcholine occurred in layers which were encountered during each penetration at successive depths of approximately 500 μ. The cells were generally characterized by a rapid spontaneous discharge, small spike amplitude and close proximity to several other cells of similar type. These features are characteristic of the granule layer cells, and our evidence indicates that cells in the molecular layer and superficial Purkinje cells were not affected by acetylcholine, though the latter were identified only on the basis of depth, amplitude of spikes and a slow intermittent spontaneous discharge. A higher proportion of cells in the deeper granule cell layers responded to acetylcholine. Histochemical techniques have been employed to define the distribution of cholinesterase in the cat cerebellar cortex[8]. The enzyme is restricted to the granule cell layers and it may be of significance that the deeper granule cell layers stain more densely.

Acetylcholine-sensitive cells have been found in all areas of the cerebellum thus far investigated, including the anterior vermal cortex, folium vermis, tuber vermis, pyramis, paramedian lobule, Crus I and Crus II.

Further investigations into the pharmacology of these cells showed that they are excited by a range of cholinomimetic agents, including in descending order of potency carbamylcholine, acetyl-β-methylcholine (acetylcholine), nicotine, and arecoline. The Figure (A) illustrates the excitant actions of glutamate, acetylcholine and nicotine on 2 cells. The unit with the smaller spike was discharging spontaneously and responded to the glutamate application. This neurone was also fired by acetylcholine and

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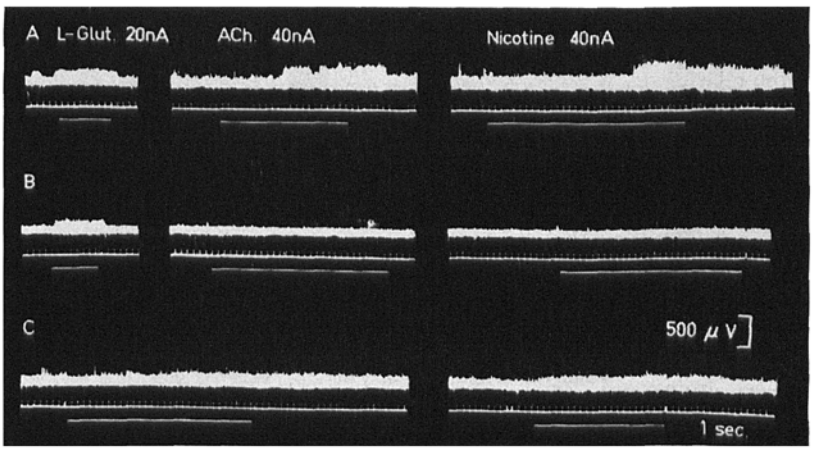

Extracellular action potentials of 2 cells located at a depth of 2.17 mm in the tuber vermis of a cat. White lines indicate times during which substances were released. A, Records demonstrating the discharge patterns of both neurones during the application of glutamate, acetylcholine and nicotine from different barrels of the same compound pipette. B, Records taken 30 sec, 2 min and 3 min respectively after the end of a 90 sec application of dihydro-β-erythroidine (DHE) by a current of 120 nA. C, A further series of records 15 and 17 min after DHE showing partial recovery to the actions of acetylcholine and nicotine.

nicotine with a short latency and then a second cell which presumably had a higher threshold commenced to discharge. Dihydro-β-erythroidine (DHE) was then applied by a current of 120 nA for 90 sec. The Figure (B) recorded shortly after the cessation of this application demonstrates that while glutamate excitation was unimpaired the cell was now no longer discharging spontaneously, and was not excited by either acetylcholine or nicotine. 15 min later (Figure C) the cell with the smaller spike had resumed discharging spontaneously and could be excited by acetylcholine and nicotine. The second cell remained in an inexcitable state for a further 60 min.

Atropine and to a lesser extent gallamine, mecamylamine and *d*-tubocurarine also antagonized the excitant actions of acetylcholine. The anticholinesterases, neostigmine and BW. 284C51 exhibited powerful excitatory actions, in many instances being almost as active as acetylcholine. Such a marked excitant action was not shown by these compounds on either Renshaw or Betz cells[7,9] and it is tempting to suggest that this action may indeed have been due to inactivation of cholinesterase, rather than a direct effect.

In conclusion, therefore, it is clear that acetylcholine has a marked excitant action on cells in the cerebellar cortex of the cat. It seems likely that these cells belong to the granule cell layer. The pharmacological findings described indicate the possibility that acetylcholine may be mimicking the action of a naturally occurring synaptic transmitter. That acetylcholine or related compound may have a functional role in synaptic transmission is suggested by the pronounced potentiating action of anticholinesterase drugs and depressing action of DHE on spontaneous firing.

Résumé. Nous avons examiné directement 562 cellules du cervelet du chat par la méthode iontophorétique. Dans les chats anesthétisés par Dial, 9% des cellules étaient excitées par l'acétylcholine ou les substances cholinomimétiques, mais dans les chats anesthétisés par pentobarbital, la proportion était de 39%. La distribution des cellules responsives correspondait à la distribution des cellules de la couche granulaire. L'action de l'acétylcholine et l'activité spontanée étaient bloquées par la dihydro-β-erythroïdine.

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The Distribution of Cholinesterase in Cat Cerebellar Cortex

The experiments described in the preceeding paper[1] have made it necessary to extend previous studies on cerebellar cholinesterase[2–4], which dealt with vertebrate species other than the cat. Moreover, histochemical studies may be used as valuable adjuncts to such pharmacological studies, in localizing both cells and fibre tracts which may be involved in cholinergic synaptic transmission[5,6]. A detailed study of the distribution of cholinesterase in the rabbit, rat and guinea-pig[4] has led to the postulate that transmission at synaptic junctions between granule cell axons, Purkinje cell dendrites and stellate cells in the molecular layer is mediated by acetylcholine, as is transmission between granule cells and Golgi cells in the granule cell layer. Possession of similar detailed information for the feline cerebellar cortex would obviously be of considerable value in any pharmacological investigation.

Preliminary experiments were carried out on homogenized cerebellar cortical tissue to ascertain the relative proportions of acetylcholinesterase and pseudocholinesterase and also to determine what concentration of the specific inhibitors for the two enzymes should be used in the histochemical procedures. The cats were prepared by opening up the thoracic cavity after anaesthetization with pentobarbital sodium and perfusing 400 ml of 0.9% saline solution through the ascending aorta after occlusion of the descending aorta. In cats that were to be used for histochemical procedures this was followed by 400 ml of 10% formol saline to fix the brain *in situ*. A cerebellum was only accepted for further study if the perfusion had been completely successful in removing all blood from blood vessels in the tissue, thus avoiding any complications arising from erythrocyte and plasma cholinesterase.

Table illustrates the relative rates of hydrolysis of four substrates by cat cerebellar cortex cholinesterase and the degree of inhibition of this enzyme by three inhibitors. The enzyme hydrolyses acetyl-β-methylcholine but not

Substrate	Relative activity	Inhibitor	Pre-incubation time (min)	I_{50} (μM)
Acetylcholine	100	284 C 51	–	1.8×10^{-2}
Acetyl-β-methylcholine	50	*iso* ompa	45	10^{3}
Butyrylcholine	20	DFP	120	6.3×10^{-1}
Benzoylcholine	2.5			

Substrate and inhibitor specificity of cat cerebellar cholinesterase. The cortex from cat cerebellum was homogenized in Krebs bicarbonate buffer and diluted to 4 mg wet weight per ml. The homogenate was pre-incubated at room temperature with *iso* ompa and DFP for the times indicated. All subsequent measurements were carried out manometrically at 37°C, using acetylcholine as substrate.

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