Exclusively inhibitory action of iontophoretic acetylcholine on single neurones of feline thalamus

IN most regions of the mammalian brain only a small percentage of the neurones located with multi-barrelled micropipettes are inhibited by iontophoretic acetylcholine (ACh). Indeed, the much more frequent occurrence of cells excited by rather small doses of acetylcholine (see ref. 7 for review), together with the finding that both the inhibitory and excitatory actions of ACh are often antagonised by the same pharmacological agents, has led to the suggestion that the inhibitory action of ACh is mediated indirectly through the excitation of neighbouring cholinoceptive interneurones whose inhibitory transmitter is a substance other than ACh[1,2]. Several workers[4–6] have tried to overcome this difficulty by working in the most superficial layers of the cerebral cortex where a strong inhibitory action of ACh predominates and ACh excitation has seldom been reported. We wish to draw attention to the possibility that the nucleus reticularis of the cat thalamus may well prove to be an appropriate area in which to study the depressant action of ACh on central mammalian neurones since almost every cell encountered proved to be inhibited by a short, low amplitude pulse of ACh.

Four cats anaesthetised with a mixture of halothane (0.5%), nitrous oxide (65%) and oxygen were placed in a stereotaxic frame and burr holes prepared in both sides of the skull at the appropriate stereotaxic coordinates. Through each burr hole a single track was made using a freshly filled five barrelled micropipette which contained one barrel filled with 4 M NaCl for single unit recording, another with 1 M NaCl for current and current balancing controls and two drug barrels containing 1M AChCl and 1 M sodium glutamate. In this preliminary study no other drugs or putative inhibitory transmitters were present in the microelectrodes. The coordinates of the electrodes were AP9.5 and L7.0 or 8.0 and recording began at H7.0 and continued until H0.0 (ref. 9). After a single microelectrode track had been made on each side of the brain the animals were more deeply anaesthetised with pentobarbitone, killed by perfusion fixation, and the brains prepared for histological analysis.

In Fig. 1*b* five microelectrode tracks, made in four cats on the stereotaxic coordinates AP9.5 and L7.0 or 8.0, are represented schematically by vertical lines to show the depth of the neurones which were either inhibited (●) or excited (×) by small iontophoretic applications of ACh as the microelectrodes transversed first the nucleus reticularis and then entered the ventrobasal complex of the thalamus. Track 1, which lay within the thickness of a single histological section and from which the line drawn in Fig. 1*a* was traced, penetrated only the nucleus reticularis and located only cells inhibited by ACh. All other tracks lay slightly more medial to track 1 and penetrated both the nucleus reticularis and the ventral basal complex of the thalamus. Tracks 4 and 5 are from the same cat. In track 4 the results suggest that the electrode re-entered the nucleus reticularis at the lower limit of its descent and again located cells inhibited by ACh. During the withdrawal of this electrode through the nucleus reticularis three more cells inhibited by ACh (○) were found. During a subsequent descent of the same electrode on the right side of the ca (track 5), the depth distribution of cells inhibited an excited by ACh was almost identical to that seen in trac 4. This experiment eliminates the possibility that the chang from ACh-evoked inhibition to excitation reflects a pro gressive deterioration in either the health of the anima or the effectiveness of the microelectrode and its content The oscillographs in Fig. 2 illustrate the changes in singl unit activity of two neurones located in track 4 durin iontophoretic applications of ACh. The first cell locate in the nucleus reticularis at the approximate stereotaxi coordinate H4.41 shows the typical spontaneous activity o all cells located in this region and was strongly inhibited b ACh. The second neurone which lay approximately 2.43 mm deeper than the nucleus reticularis shows the typical excita tion evoked by iontophoretic ACh applied to a majority o neurones in the ventrobasal thalamus[10]. The ACh-evoke inhibition could not be mimicked by the passage of large amounts of positive current through the NaCl barrel o the microelectrode and was only marginally reduced in intensity when the current through the ACh barrel wa balanced by passing a negative current of equal magnitud through the NaCl barrel. Of 74 cells found to be inhibited in the five tracks shown schematically in Fig. 1*a* (cell less than 50 μm apart and showing the same response to ACh are represented by a single symbol) 17 were inhibited when the retaining current on the ACh barrel (25 nA) wa reduced and 57 more by currents of less than 40 nA (33 ± 3 nA).

In addition, the inhibition evoked by the release of ACh by relatively low current applications had a shorter latency of onset than the excitations evoked by either the same or larger currents from the same microelectrode during its descent through the deeper nuclei of the thalamus. The observations that the onset of ACh-evoked inhibition is of

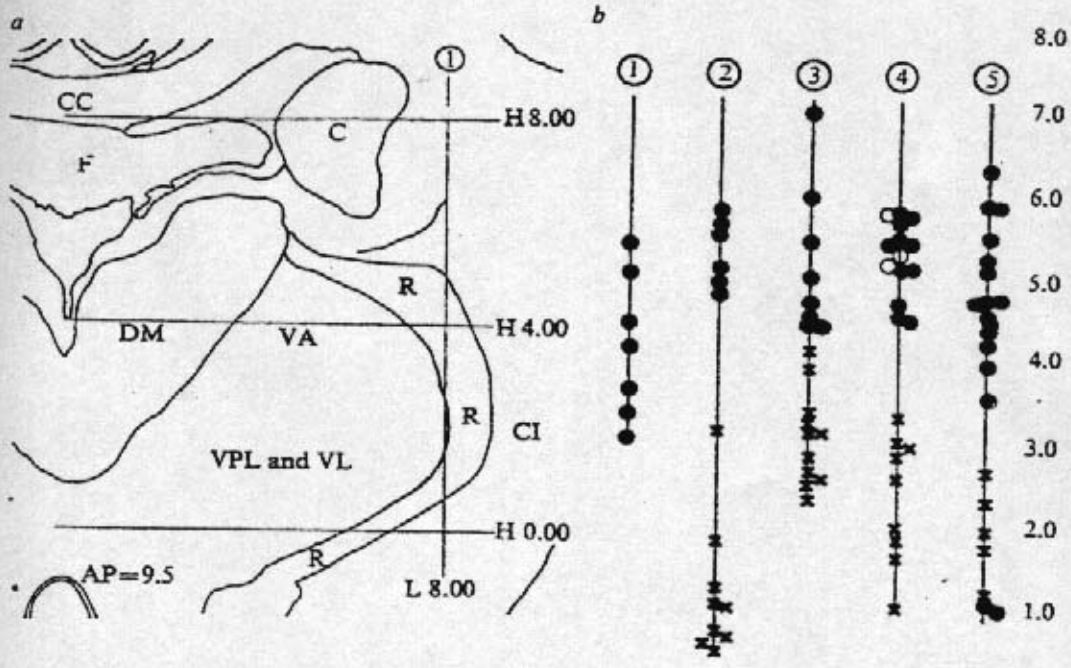

Fig. 1 Schematic drawings to show how cells located within the nucleus reticularis were inhibited by iontophoretic ACh and those lying deeper in the ventrobasal complex of the thalamus excited. *a*, From the histological section which contained the microelectrode track represented by line 1 in *b* shows line L8.00 down which this particular microelectrode passed. The five vertical lines in *b* schematically show the depth distribution of the cells which were either inhibited (●) or excited (×) as microelectrodes penetrated or were withdrawn (○) from the brain of four cats along the coordinates AP9.5 and L7.0 (2 and 3) or 8.0 (1, 4 and 5). Tracks 4 and 5 are from the left and right side of the same cat. The depths of the cells along each track were read from the micrometer of the micromanipulator drive and no attempt has been made to correct these values so that they correspond with the coordinates taken from the atlas of Snider and Niemer[9] and arbitrarily assigned to the line drawing.

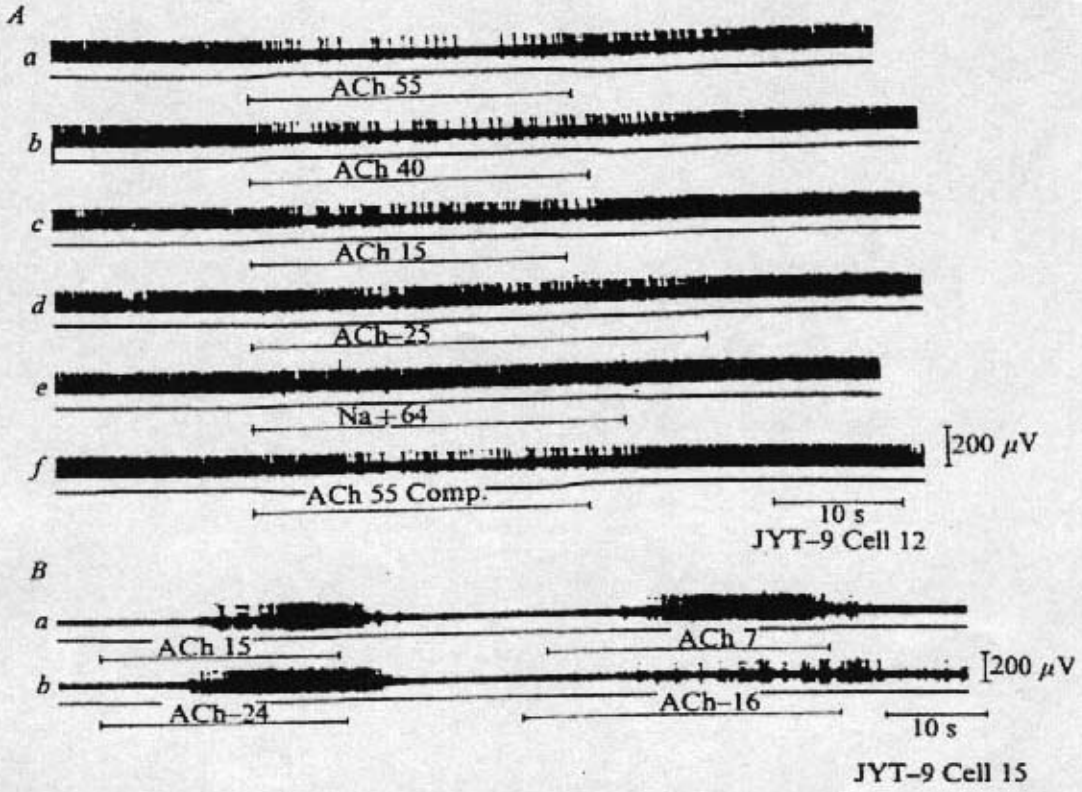

Fig. 2 Oscillographic records to show the inhibition and excitation of neurones in the nucleus reticularis and ventrobasal complex of the thalamus located in the same microelectrode track. *A*, From a cell located at the stereotaxic coordinate H4.4I showing: *a*–*e*, dose-related inhibitions evoked by progressively smaller doses of ACh released by currents from 55–15 nA; *d*, effect of withdrawing 25 nA of the 25 nA backing current; *e*, lack of effect of 60 nA positive current passed through NaCl barrel; *f*, effect of balancing the 55 nA passed through the ACh barrel by −55 nA passed through the NaCl barrel. *B*, Records *a* and *b* show excitation of a neurone located 2.43 mm deeper in the track by similar doses of ACh. Note that the delay between the onset of the ACh application and the ACh-evoked excitation in *B* was greater than that which occurred in *A* between the onset of the ACh pulse and the ACh-evoked inhibition.

shorter latency than ACh-evoked excitation are in keeping with the view of Krnjević[1], who has suggested that ACh-evoked excitation in the cerebral cortex is often preceded by a period of inhibition. Since in the nucleus reticularis ACh-evoked inhibition has a shorter latency than the excitation evoked by larger currents in the ventrobasal complex, it is difficult to believe that ACh-evoked inhibition could be mediated by the excitation of an intervening cholinoceptive interneurone. The possibility that the inhibitory action of ACh might be mediated by a presynaptic mechanism was eliminated in part by showing ACh to be an equally effective inhibitor of cells when firing was enhanced by glutamic acid.

The finding that the action of ACh in the thalamus is excitatory in one region and inhibitory in another could be of great functional significance. For instance it now seems possible that a single cholinergic pathway could excite neurones of the ventrobasal complex and inhibit neurones of the nucleus reticularis. This 'push–pull' arrangement is in keeping with the neurophysiological data of Schlag and Waźak[12] and Massion and Rispal-Padel[13], which has led to the suggestion that the firing of the neurones of the ventrobasal complex is modulated by inhibitory interneurones whose cell bodies lie within the nucleus reticularis. This idea is contrary, however, to Jasper's[14] original hypothesis which supposed that the nucleus reticularis was the 'final relay' nucleus of the ascending excitatory pathway involved in 'cortical arousal'. Moreover, modern anatomical studies of the thalamus have not been able to confirm the presence of a pathway from the nucleus reticularis to the cortex and indeed show the majority of the neurones of this nucleus to project to the ventrobasal complex of the thalamus[14–16]. Thus Jasper's original view[14] that the nucleus reticularis is intimately involved in cortical arousal can only be retained by assuming that the neurones of the nucleus reticularis are inhibited by the ascending cholinergic pathway and thus bring about cortical arousal indirectly through disinhibition of the neurones of the more medial nuclei of the thalamus. Interestingly enough, similar physiological findings[17,18] have led Krnjević[1] to suggest that the ACh-inhibited cells in the upper layers of the cerebral cortex bring about disinhibition of ACh-excited neurones lying much deeper in the cerebral cortex. An analogous situation exists in the nervous system of *Aplysia*, in which the firing of a single cholinergic neurone has been shown to excite one cell and inhibit another[19,20].

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