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ACETYLCHOLINE-SENSITIVE CELLS IN THE CAUDAL MEDULLA OF THE RAT: DISTRIBUTION, PHARMACOLOGY AND EFFECTS OF PENTOBARBITONE

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1 The distribution of cholinoceptive and non-cholinoceptive cells in various nuclei of the caudal medulla of the rat is described.

2 The nature of the responses of cells of the paramedian reticular nucleus and of the perihypoglossal nuclei to electrophoretically applied acetylcholine (ACh) was investigated.

3 In unanaesthetized decerebrate preparations ACh responses were usually of a 'fast onset-fast offset' nature. Dihydro-β-erythroidine was a more effective antagonist than atropine.

4 In rats anaesthetized with barbiturate nearly all the ACh responses showed a slower onset and prolonged action. Atropine was the more effective antagonist.

5 The synaptic responses of cells of the paramedian reticular and perihypoglossal nuclei to stimulation of glossopharyngeal, superior laryngeal, lingual and hypoglossal nerves were investigated. It is concluded that ACh does not mediate the responses at the level of these nuclei.

Introduction

Previous reports concerning acetylcholine (ACh)-sensitive cells in the medulla of the cat (Salmoiraghi & Steiner, 1963; Bradley, Dhawan & Wolstencroft, 1966; Avanzino, Bradley & Wolstencroft, 1966; Yamamoto, 1967) and of the rat (Bradley & Dray, 1972, 1973) contain little data on the synaptic connections of these cells or on their location in different nuclear groups. However, in the cat Avanzino *et al.* (1966) found that ACh excited almost all those cells in the region of the paramedian reticular nucleus which were antidromically activated from the cerebellum.

Excitation of medullary reticular cells by ACh has been reported in the rat anaesthetized with barbiturate and in unanaesthetized animals (Bradley & Dray, 1973) and it was suggested that the short latency excitation observed in decerebrate rats may subserve a synaptic function.

In the present experiments an attempt was made to relate excitation of identified rat medullary cells by ACh to a synaptic input. In addition, the nature of the response to ACh and the specificity of nicotinic and muscarinic antagonists in rats anaesthetized with barbiturate were found to be different from those in decerebrate preparations.

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Methods

Rats were either anaesthetized with pentobarbitone sodium 45 mg/kg intraperitoneally, supplemented with intravenous injections of a 10 mg/ml solution, or were decerebrated under halothane anaesthesia. The latter involved electrolytic coagulation of the midbrain followed by suction of cerebral tissue rostral to the coagulated area. Both common carotid arteries were tied to minimize blood loss during this procedure. Anaesthesia was discontinued after decerebration. All animals were artificially ventilated with humidified oxygen following muscle relaxation with gallamine triethiodide.

The brain stem was approached ventrally as previously described (Duggan, Lodge & Biscoe, 1973). The following nerves were prepared for stimulation as a means of identifying cell groups in the medulla: superior laryngeal, recurrent laryngeal, glossopharyngeal, hypoglossal and lingual nerves.

Compounds were administered electrophoretically from the outer barrels of seven barrel micropipettes. These compounds, together with

their concentrations and pH, were: L-glutamate Na (Koch-Light), 0.5 M pH 8; DL-homocysteate Na (Koch-Light), 0.2 M pH 8; acetylcholine bromide (BDH), 0.5 or 1.0 M pH 3.3; acetyl-β-methylcholine bromide (Koch-Light), 0.5 M pH 3.7; atropine sulphate (Koch-Light), 10 mM in 165 mM NaCl pH 4.6; dihydro-β-erythroidine (Merck, Sharpe & Dohme), 0.1 M pH 4.6; pontamine sky blue (G.T. Gurr), 2% in 0.5 M Na acetate pH 7.7. A retaining voltage of 0.5 V was routinely applied to each barrel except that containing the dye (see Lodge, Caddy, Headley & Biscoe, 1974); however, lower retaining voltages were frequently used on barrels containing the excitants so as to minimize the latency to the onset of their actions from the beginning of the application of an ejecting voltage.

Action potentials were recorded with the 4 M NaCl filled centre barrels of the micropipettes, and by conventional techniques the firing rates of single cells were displayed continuously on a potentiometric pen recorder. The synaptic activation of cells was studied quantitatively by preparing poststimulus histograms using a small computer (Biomac 500).

During the course of each experiment, physiological landmarks, e.g. the field potential from antidromic activation of hypoglossal motoneurones, as well as the usual micromanipulator readings, were used as guides to the position of the microelectrode tip. Pontamine sky blue was ejected at stereotaxically defined recording sites, and the resultant blue spots were found by cutting 25 or 50 μm frozen sections of the formalin-fixed brain stem. Thus by a combination of micromanipulator readings and histologically identified dye marks, the locations of most of the cells investigated were plotted on outline drawings of serial brain stem sections.

Results

The present study describes the location of ACh-sensitive cells in various nuclei in the caudal medulla of the rat. An attempt was made to correlate sensitivity to ACh with synaptic inputs; also the effect of ACh antagonists on synaptic transmission was tested on cholinoceptive cells. During the course of this investigation a previously undescribed difference in the pharmacology of cholinoceptive cells in the paramedian reticular and perihypoglossal nuclei was found between decerebrate and barbiturate-anaesthetized rats.

(1) Distribution of cholinoceptive cells in the medial part of the caudal medulla

Cells were located by their spontaneous discharge, by their response to the ejection of ACh or an excitant amino acid, or by their response to stimulation of the prepared cranial nerves. The distribution of 109 such neurones in tracks not more than 0.9 mm lateral to the midline is shown in Figure 1. No attempt was made to estimate the proportions of cholinoceptive and non-cholinoceptive cells in any particular area as the problems of sample bias make such estimates inaccurate. However, certain generalizations about the responses to ACh of various cell groups in this region can be made.

(a) Paramedian reticular and perihypoglossal nuclei A large proportion of neurones in these areas project to the cerebellum (Brodal & Torvik, 1954). In three experiments such cells were identified by juxtafastigial cerebellar stimulation which caused both antidromic and synaptic excitation; examples of such responses are shown in Figures 4a & b. The short latency (1.1 ms) and the high following frequency (500 Hz) suggest that the spikes in Figure 4a and the first in Figure 4b were antidromically evoked whereas the second spike in Figure 4b probably resulted from orthodromic activation. In all other experiments the paramedian reticular nucleus and the perihypoglossal nuclei were identified only histologically. Many cells in this area were cholinoceptive; it seemed that such cells were sampled more readily in decerebrate rats, for of the 99 cholinoceptive neurones studied 70 were found in 10 decerebrate, and 29 in 11 barbiturate anaesthetized preparations.

(b) Hypoglossal nucleus Throughout this series of experiments many tracks of the electrode were made through the hypoglossal nucleus, the antidromic field of which served as a useful reference to electrode trip position (see methods section). No cholinoceptive cells were ever found.

(c) Inferior olivary nucleus The actions of ACh in this nucleus have been described in a separate communication (Duggan, Lodge, Headley & Biscoe, 1973). Neurones responded to ACh with a marginal increase in firing rate.

(d) Nucleus of the solitary tract Neurones in this nucleus were defined as those activated from a single stimulus to the glossopharyngeal or superior laryngeal nerve with a latency of between 2 and 5 ms. Such responses were usually superimposed on a large negative field potential (Fig. 4d) which helped to define the area. None of the five cells tested was cholinoceptive.

(e) More laterally located neurones Neurones of the nucleus ambiguus, antidromically evoked from

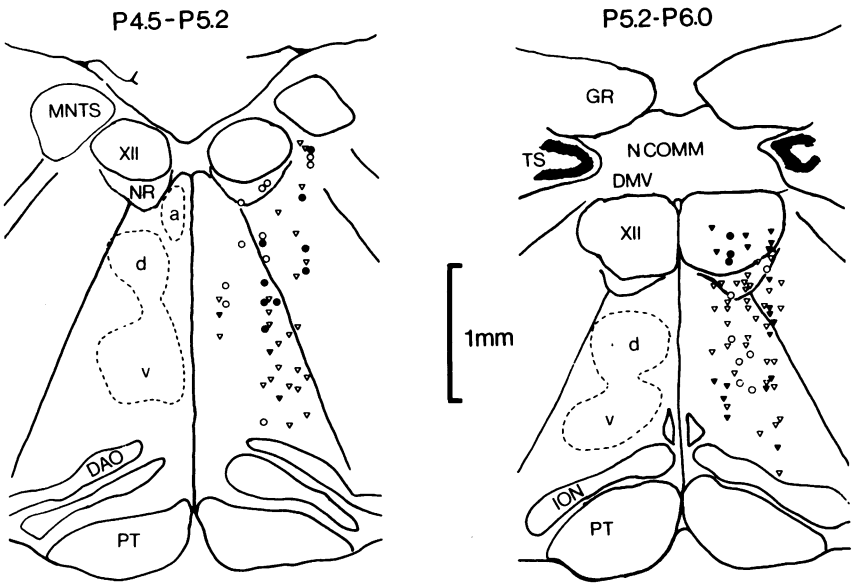

Figure 1 Distribution of cholinoceptive and non-cholinoceptive cells in the region of the paramedian reticular and perihypoglossal nuclei of the rat. The positions of cholinoceptive (open symbols) and non-cholinoceptive (closed symbols) neurones in both barbiturate anaesthetized (circles) and decerebrate (triangles) preparations, were projected onto the appropriate line drawing by reference to stereotaxic coordinates, pontamine sky blue marks and the antidromically evoked hypoglossal field depth profile. The figures above the section give the inclusive antero-posterior coordinates of each. a,d,v = accessory, dorsal and ventral parts of the paramedian reticular nucleus as proposed for the cat by Brodal & Torvik (1954); DAO = dorsal accessory olive; DMV = dorsal motor nucleus of the vagus; GR = gracile nucleus; ION = inferior olivary nucleus; N COMM = commissural nucleus of Cajal; NR = nucleus of Roller; PT = pyramidal tract; TS = solitary tract; XII = hypoglossal nucleus; MNTS = medial nucleus of the solitary tract.

the recurrent laryngeal nerve, were not cholinoceptive. Some physiologically unidentified neurones in the surrounding reticular formation were found to be excited by ACh.

No cells were studied in the sensory nucleus of the trigeminal nerve, the dorsal column nuclei, the motor nucleus of the vagus or in the commissural nucleus of Cajal.

(2) The nature of the response of neurones in the paramedian reticular and perihypoglossal nuclei to electrophoretically applied acetylcholine; comparison of decerebrate and barbiturate-anaesthetized preparations

The actions of ACh on central neurones of the cat fall broadly into two groups; a 'fast onset-fast offset' action typified by the nicotinic response of spinal Renshaw cells (Curtis & Ryall, 1966), and a slow onset-prolonged action as seen at the muscarinic receptors of cerebral cortical neurones (Krnjević & Phillis, 1963; Crawford & Curtis, 1966). In the present study a 'fast action' is defined as one in which peak firing frequency was reached within 10 seconds.

As the decerebrate and the barbiturate-anaesthetized animals differed in the types of response they will be described separately.

(a) Decerebrate preparations In the 10 decerebrate rats, 35 of 70 cholinoceptive cells showed that 'fast' type of response to ACh, whereas with 19 the 'slow' response was observed. On the remaining 16 neurones ACh had an excitant effect which had a variable latency and so was difficult to classify into these two groups. No depressant action either on spontaneous firing or on the firing in response to the ejection of L-glutamate was observed apart from that considered to be due to cationic current.

The nature of the ACh response of 17 neurones was further examined by ejecting dihydro-β-erythroidine (DHβE; 6-30 nA) and/or atropine (8-52 nA) during the alternate ejection of acetylcholine and L-glutamate or DL-homocysteate (DLH). Of the seven cells on which the actions of both DHβE and atropine were tested, four showed selective ACh antagonism by DHβE but not by atropine, three showed antagonism by both substances whereas none

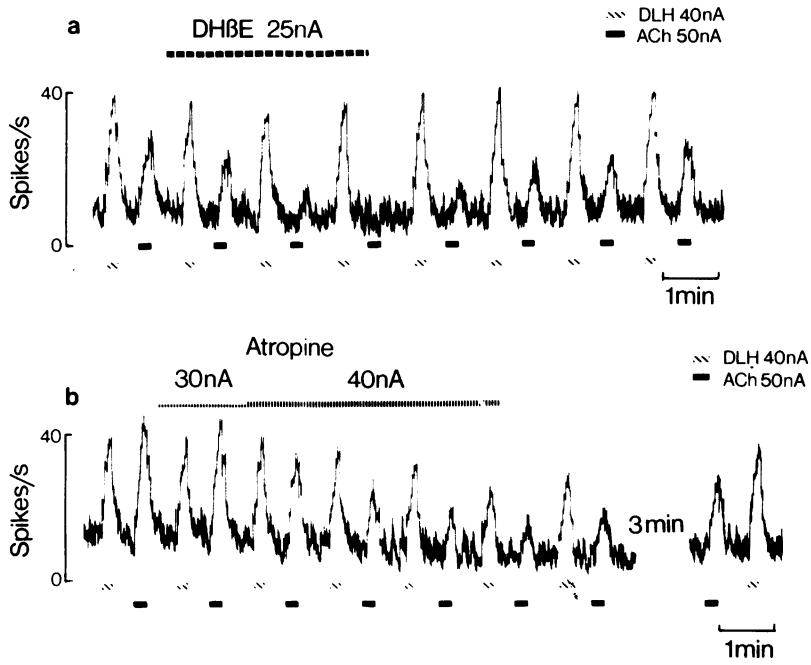

Figure 2 Effects of dihydro-β-erythroidine (DHβE) and atropine on acetylcholine (ACh) and DL-homocysteate (DLH) excitant actions on a perihypoglossal neurone in a decerebrate rat. The ratemeter records of (a) and (b) show consecutive tests on this cell which responded quickly to the ejection of ACh (50 nA for 15 seconds). The time constant of the ratemeter was 1 second. During the recovery from atropine 3 min of the trace have been omitted.

showed antagonism by atropine alone. On a further nine cells, DHβE alone was tested; reversible reduction of the ACh response alone was seen in five cells, and with three the action of ACh was reduced more than that of L-glutamate but full recovery was not observed. On the remaining cell, the only one of the 'slow' type, the ACh response was unaffected by DHβE (20 nA).

On one other cell atropine alone (up to 52 nA) was tested and no effect was observed until the responses to both excitants were reduced. Such high currents of atropine often resulted in reduction in spike amplitude (Curtis & Phillis, 1960).

The records of Figure 2 were obtained from a cell in the perihypoglossal nuclei. The action of acetylcholine was 'fast', being comparable in onset and decline to that of DLH. On this cell DHβE (25 nA) reversibly abolished the action of ACh without affecting that of DLH (Figure 2a), whereas atropine had no effect on the response to ACh until the actions of both excitants were reduced (Figure 2b).

Thus most cells in decerebrate preparations, had predominantly 'fast' ACh responses which on the basis of their sensitivity to the ACh antagonists, appeared to be mediated mainly by receptors of the nicotinic type.

(b) Rats anaesthetized with barbiturate In 11 such preparations ACh had a 'slow' action on 25 cells and a clearly 'fast' action on only 2 out of 29 cholinoceptive neurones studied. Not only was the time course of the responses longer than those seen in decerebrate preparations but ACh was a less potent excitant, relative to glutamate. In decerebrate preparations, the currents of ACh (20-60 nA, mean 44 nA) were usually similar to those of nearly equieffective currents of L-glutamate (16-70 nA, mean 39 nA), whereas in rats anaesthetized with barbiturate ACh ejecting currents (40-100 nA, mean 66 nA) needed to be two to three times as high as those of L-glutamate (8-56 nA, mean 26 nA) in order to produce excitant responses of a similar magnitude.

In rats anaesthetized with barbiturate, atropine (1-60 nA) was more effective as an ACh antagonist than was DHβE (8-180 nA). With all five cells tested with both antagonists, atropine selectively reduced the action of ACh whereas DHβE only did so on one. Atropine alone was tested on a further five cells and on only one was ACh antagonism not

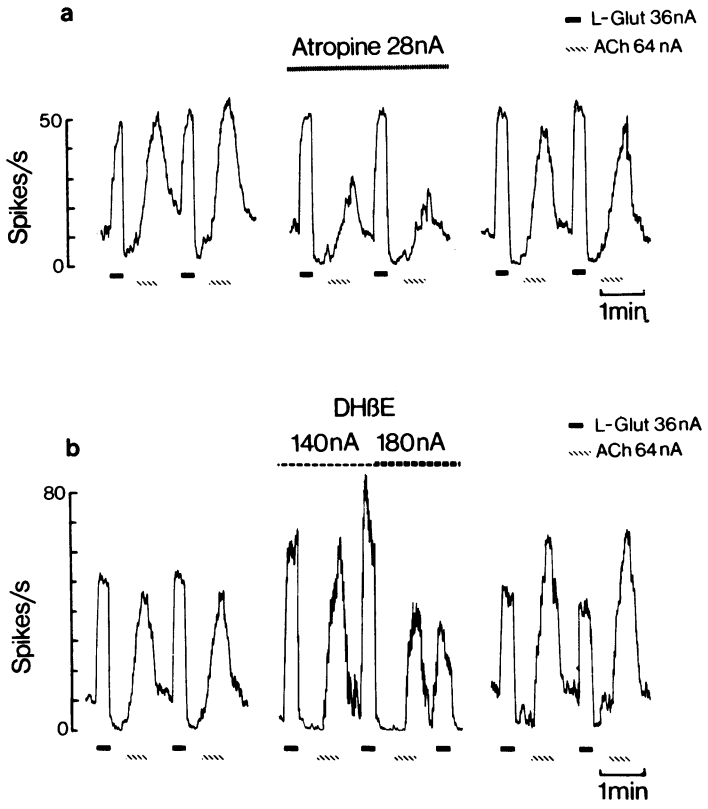

Figure 3 Effects of atropine and dihydro-β-erythroidine (DHβE) on acetylcholine (ACh) and L-glutamate (L-Glut) responses of a paramedian reticular neurone in a rat anaesthetized with barbiturate. This neurone responded only slowly to ACh requiring 30 s at 64 nA to reach a near-plateau firing rate. The action of L-glutamate (64 nA 15 s) was more rapid. Ratemeter time constant 0.3 s. (a) Is divided into three parts. The first shows control responses after which atropine was ejected with currents of 8 nA for 3 min and 16 nA for 1.5 minutes. The second part shows responses recorded whilst the atropine current was raised to 28 nA. The third part was taken 10 min after cessation of the atropine current. In (b), after the control response, the DHβE current was increased stepwise from 10 nA to 20, 30, 40, 60, 80 and 140 nA over a period of 15 min (not shown). The second part of the record shows the effect of the last part of 140 nA DHβE and the response when the current was raised to 180 nA. Recovery is shown 17 min after cessation of the DHβE current.

observed. On three other cells tested with DHβE alone, there was either no effect (two cells) or both ACh and glutamate responses were reduced (one cell).

Results from one rat anaesthetized with barbiturate are illustrated in Figure 3. Pontamine sky blue marking showed this cell to be located in the dorsal part of the paramedian reticular nucleus. The excitant action of ACh was slow in onset and prolonged by comparison with that of L-glutamate. An atropine current of 28 nA (Figure 3a) preferentially reduced the action of ACh; recovery is illustrated. On this same cell DHβE was ejected with increasing currents up to 180 nA; initially the responses to both excitants were increased (see 140 nA trace, Figure 3b), and then both were reduced in parallel. This effect has been seen on a number of occasions in the brain stem (see also section 4 below). The frequent occurrence of similar increases in the responses of rat Renshaw cells to excitants during the ejection of DHβE, has led us to conclude that this effect is related to the known direct excitant action of this drug (e.g. Curtis & Ryall, 1966).

The responses of cells in preparations anaesthetized with barbiturate thus show a marked difference from those in the decerebrate animals both in the nature of the response to ACh and in the susceptibility of this response to atropine and DHβE. The results suggest a preponderance of ACh receptors of the muscarinic type in the anaesthetized rats.

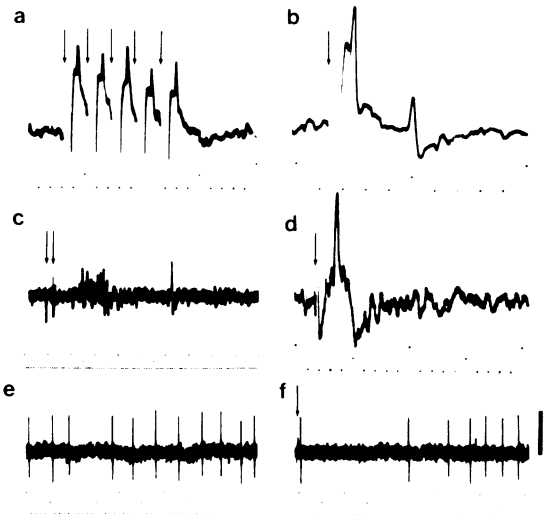

Figure 4 Activation of neurones in the area of the paramedian reticular nucleus. (a) Responses of a cholinoceptive cell just ventral to the hypoglossal nucleus to tetanic stimulation (500 Hz, 100 μA) near the contralateral fastigial nucleus. (b) Same cell as (a) showing response to a single shock (100 μA) near the ipsilateral fastigial nucleus. (c) Response of a cholinoceptive neurone in the ventral part of the paramedian reticular area to 2 stimuli (300 Hz, 100 μA) to the glossopharyngeal nerve. (d) Response of a non-cholinoceptive neurone in the region of the nucleus of the solitary tract to a single stimulus (1.5 V) to the glossopharyngeal nerve. (e and f) Slow sweep traces showing the steady firing pattern of a cell in the dorsal part of the paramedian reticular nucleus (e) during the ejection of acetylcholine 40 nA. In (f), the same firing rate is interrupted by a single stimulus (80 μA) to the ipsilateral superior laryngeal nerve. The calibration bar to the bottom right represents 200 μV for (a), (b) and (c) and 100 μV for (d), (e) and (f). The sweep durations illustrated are (a) 20 ms, (b) 10 ms, (c) 100 ms, (d) 20 ms, (e and f) 500 ms.

Acetyl-β-methylcholine was tested cursorily on seven cholinoceptive cells, and in each case had an effect similar to that of ACh, whether the responses to ACh were fast (two cells, decerebrate), intermediate (two cells, decerebrate) or slow (three cells, barbiturate).

(3) The synaptic responses of cells in the region of the paramedian reticular and perihypoglossal nuclei

(a) Glossopharyngeal nerve A single stimulus to the glossopharyngeal nerve seldom affected the firing rate of cholinoceptive cells. However a brief tetanus (2-3 stimuli of 50-150 μA and 0.15 ms duration, at 300 Hz) influenced the firing of 30 of the 39 cholinoceptive cells tested in decerebrate animals. Twenty cells were excited and 10 were inhibited.

The excitation usually consisted of a short train of spikes (Figure 4c). The latency of activation varied for any one cell but when poststimulus histograms were prepared the time of greatest probability of firing was normally seen to be between 15 and 30 milliseconds. This latency is greater than that of cells of the nucleus of the tractus solitarius evoked by a single shock to the glossopharyngeal nerve (Figure 4d). Whilst most cells of the paramedian region responded with a single burst of firing some followed this with a second, more prolonged burst.

The inhibitions were usually prolonged, often lasting several hundred milliseconds. An example is illustrated in Figure 4e & f; in this instance a single stimulus was sufficient to cause a prolonged inhibition. No intracellular or pharmacological studies to determine the nature of such inhibition have been attempted.

In animals anaesthetized with barbiturate, only two of eight cholinoceptive cells tested were activated by a brief tetanus to the glossopharyngeal nerve; one was inhibited.

In both types of preparation, stimuli to the glossopharyngeal nerve could also affect non-cholinoceptive cells of this region. The latency and the character of the evoked responses were similar to those of the cholinoceptive cells.

(b) Superior laryngeal nerve In decerebrate preparations, with stimulation parameters similar to those used with the glossopharyngeal nerve, 20 out of 25 cholinoceptive cells tested were influenced by a single stimulus to the superior laryngeal nerve; 11 were excited and 9 were inhibited. The latency of activation was similar to that following glossopharyngeal stimulation. Of the 22 cells on which the effects of stimili to both glossopharyngeal and superior laryngeal nerves were recorded, 17 responded to both with the same type of firing pattern whilst opposite effects were observed on 5 cells.

In animals anaesthetized with barbiturate, only one of eight cholinoceptive cells tested could be evoked by superior laryngeal nerve stimulation. This cell showed a pause in firing.

(c) Lingual nerve This input was tested on only 10 neurones confined to anaesthetized preparations. Three were excited, three were inhibited and four were unaffected. The character of the evoked response was similar to that previously described for the glossopharyngeal and superior laryngeal nerves.

(d) Hypoglossal nerve No cells were activated or inhibited by either single stimuli or short tetani to the hypoglossal nerve. Although small field potentials were evoked in this region, these were always referable to the adjacent hypoglossal nucleus.

(4) The effect of acetylcholine antagonists on the synaptic activation of cholinoceptive cells by stimulation of the glossopharyngeal nerve

The pattern of activity following glossopharyngeal nerve stimulation was similar with both cholinoceptive and non-cholinoceptive cells. However, in decerebrate rats only, the possibility that acetylcholine mediates this synaptic excitation was tested further on nine cholinoceptive neurones by ejecting DHβE (10-40 nA; eight cells) and atropine (10-50 nA; three cells) whilst studying the probability of firing following nerve stimulation; poststimulus histograms were prepared concurrently with ratemeter records which plotted the responses to ACh and L-glutamate.

On none of the cells tested did DHβE reversibly decrease synaptic as well as ACh excitations. With five cells, higher currents of DHβE than those required to block the action of applied acetylcholine increased the responses both to glossopharyngeal nerve stimulation and to locally administered L-glutamate; this was probably due to a direct excitant effect by DHβE (see section 2b). With the three other cells no significant effect on synaptic responses was observed during the ejection of DHβE.

Of the three cells tested with atropine two showed a reduction in the synaptic response, but this was accompanied by a parallel reduction in the excitations by both L-glutamate and acetylcholine. No effect was seen on the third cell.

Discussion

The observation that cholinoceptive cells are frequently found in the region of the paramedian reticular nucleus parallels the finding of Avanzino *et al.* (1966) in the cat. However, there are no comparable data from any species on cells in the other nuclear groups studied.

In the present study it has been possible to group the ACh responses into two classes, analogous to the type I and II responses seen in cats by Bradley & Dray (1973). Certain technical complications are associated with typing neuronal responses on the basis of latency, since such latency is determined by several experimental parameters, as was originally suggested by del Castillo & Katz (1957). However, we do not think that inconsistencies of retaining and/or ejecting currents can explain the present findings, largely because short latency excitation was associated with a rapid decline at the termination of the ejecting current, and long latency excitation with a prolonged action. If the longer latency excitation was caused by excessive retaining currents, then one would expect the excitation to reverse rapidly at the end of the ejection period.

Bradley & Dray (1973) found that responses to ACh in decerebrate animals were predominantly of a fast nature whereas in preparations anaesthetized with barbiturate the proportion of slow responses was increased. No antagonists were used and hence the authors were unable to ascribe the responses to nicotinic or muscarinic receptors. In the present study the 'fast' ACh responses of the decerebrate rats were predominantly DHβE-sensitive, whereas the 'slow' responses of the rats anaesthetized with barbiturate were more often atropine-sensitive. It thus seems possible to classify the action of ACh on brain stem neurones in terms of ACh receptor type. The tests performed with acetyl-β-methylcholine would tend not to substantiate this division of receptor type, but in view of the small number of cells examined and the lack of tests with other cholinomimetics, no conclusions can be drawn other than those based on the actions of the ACh antagonists which were used. Such conclusions do of course depend on the specificity of the actions of these antagonists. In view of their specific action on different cells it does seem likely that they were antagonizing different receptor types; this is in contrast with results on Renshaw cells of rats (Biscoe, Headley & Lodge, 1974).

It seems clear from these results that the nicotinic action of acetylcholine, which was more frequently observed in decerebrate preparations, was reduced by barbiturate anaesthesia. That this was a local action of the barbiturate is likely in view of the action of electrophoretically applied pentobarbitone in rats anaesthetized with urethane. Thus both Bradley & Dray (1973) studying short latency ACh excitation, and Lodge & Martin (unpublished) who specified this excitation as being DHβE-sensitive, found that electrophoretically applied pentobarbitone reduced responses to ACh more than those to L-glutamate or DL-homocysteate.

It is however not clear whether electrophoretically or systemically administered pentobarbitone was altering the nature of the nicotinic receptor to a less effective muscarinic type or whether it was blocking nicotinic receptors thereby revealing muscarinic receptors which would otherwise have gone unnoticed. Coupled with the latter alternative, it is possible that there were two separate populations of cholinoceptive

neurones, one with muscarinic and the other with nicotinic receptors, and that in the rat anaesthetized with barbiturate the less common muscarinic neurones were more apparent since the nicotinic receptors of the larger cholinoceptive population had been blocked by the barbiturate. Only this last possibility seems consistent with the finding that DHβE could completely abolish the action of ACh on some neurones in decerebrate preparations and also with the apparent greater ease with which cholinoceptive cells were found in decerebrate preparations.

This differential effect by pentobarbitone is an unusual finding when compared with results obtained by other workers in the cat. In the thalamus of the cat anaesthetized with barbiturate Andersen & Curtis (1964) found that DHβE specifically antagonized the action of acetylcholine whereas atropine did not; the reverse of this, however, was reported by McCance, Phillis, Tebēcis & Westerman (1968). Acetylcholine-sensitive cells of the cerebral cortex have receptors of the muscarinic type regardless of the presence or absence of barbiturates (Krnjević & Phillis, 1963). On the Renshaw cell of the cat, pentobarbitone does not reduce the responses to nicotinic or muscarinic agonists before the response to DLH is also affected (Curtis & Ryall, 1966).

The present study shows that pentobarbitone does have an action on cholinoceptive neurones of the rat paramedian reticular nucleus. In the unanaesthetized cat however, 'fast' ACh responses have not been found in this area (Bradley *et al.*, 1966; Duggan & Game unpublished). Thus any inferences as to the relevance of this effect to barbiturate anaesthesia would be premature.

Controversy exists over possible synaptic inputs to the paramedian reticular nucleus in the cat; the claim that stimulation of the sinus nerve produces short latency activation of cells in this area (Crill & Reis, 1968; Miura & Reis, 1969) has not been confirmed by others (Biscoe & Sampson, 1970; Spyer & Wolstencroft, 1971). The segment of glossopharyngeal nerve stimulated in the present study in the rat was distal to its junction with the sinus nerve. Further, stimulation was usually associated with a rise in blood pressure. Hence, it is unlikely that impulses in fibres of the sinus nerve were responsible for the long latency activation of cells of the paramedian reticular nucleus. Similar neuronal responses were obtained following stimuli to glossopharyngeal, superior laryngeal and lingual nerves but, by comparison, no evidence was found of any synaptic input from the hypoglossal nerve. The present study, whilst demonstrating long latency inputs from these cranial nerves, does not provide any positive evidence as to the transmitters involved in these processes. However, since non-cholinoceptive as well as cholinoceptive neurones are activated in a similar manner, and since DHβE and atropine fail to influence this activation, it is unlikely that acetylcholine mediates the synaptic excitation at the level of the paramedian reticular and perihypoglossal neurones.

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