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A QUANTITATIVE STUDY OF THE EFFECTS OF CHOLINERGIC DRUGS ON CAROTID CHEMORECEPTORS IN THE CAT

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SUMMARY

1. Conflicting qualitative evidence exists concerning the effects on chemoreceptor activity of some drugs which influence the cholinergic system. Quantitative evidence has been obtained in the present study which should resolve the conflict.

2. Experiments were performed in pentobarbitone-anaesthetized cats in which the activity of chemoreceptor units in the sinus nerve was used to assess chemoreceptor reponses. The effects of drugs on responses to I.A. ACh and NaCN were determined from dose–response data obtained from several animals and expressed as mean dose ratios.

3. The chemoreceptor response to ACh was slightly inhibited by atropine, α- and β-bungarotoxin and HC-3, almost completely suppressed by mecamylamine, and markedly potentiated by physostigmine.

4. Concomitant responses to NaCN were unaffected by atropine, β-bungarotoxin, mecamylamine or physostigmine. There was a slight inhibition following α-bungarotoxin and a potentiation after HC-3.

5. The results do not support the theory that ACh is an excitatory sensory transmitter in the carotid body.

INTRODUCTION

It has long been known that acetylcholine (ACh) stimulates the carotid chemoreceptors (e.g. Cordier & Heymans, 1935; Heymans, Bouckaert, Farber & Hsu, 1936; Anichkov & Belen'kii, 1963), and in 1938 Schweitzer & Wright made the suggestion that drugs or changes in the blood which stimulate the chemoreceptor nerve endings might produce their effects by liberating ACh as the chemical intermediary. This possibility has since been explored by many workers, but the evidence which has accumulated concerning the role of ACh in chemoreceptor sensory transmission is equivocal and controversial (see reviews by Douglas, 1954; Heymans,

1955; Heymans & Neil, 1958; Anichkov & Belen'kii, 1963; Eyzaguirre & Zapata, 1968*a*; Torrance, 1968; Biscoe, 1971; Howe & Neil, 1972).

Much of the controversy has centred around the conflicting results obtained from experiments in which drugs were used to modify the response of the chemoreceptors to physiological and pharmacological stimuli. Heymans & Neil (1958) expressed the opinion 'that ACh has nothing whatever to do with the normal transmission of the chemoreceptor impulses' and went on to add: 'The reasons for the conflict of evidence concerning the effects of anticholinesterases and ganglioplegic drugs on the activity and sensitivity of the glomus nerve endings to normal or pharmacological stimuli require further clarification.'

From consideration of the literature it seemed probable that the conflict resulted from conclusions being based on qualitative pharmacological data, obtained from a variety of preparations, which being difficult to evaluate have been liable to subjective interpretation according to whether the evidence was intended to support or reject involvement of ACh in chemosensory transmission. The purpose of the present study was to try to resolve this conflict by using a preparation that provides a reliable indicator of chemosensory activity to determine objectively the effects of cholinergic drugs on the chemoreceptors.

METHODS

Experiments were performed on cats of either sex weighing between 2·0 and 4·9 kg (mean 2·9 kg).

Anaesthesia. The animals were anaesthetized with pentobarbitone sodium, 42 mg/kg I.P., supplemented approximately every 1·5–2 hr during the experiment by 10% of the initial dose administered I.V.

General. A cannula was inserted into the trachea low in the neck and both femoral arteries were cannulated, one catheter being connected to a B.P. transducer (Bell and Howell, 4–442) and the other used for withdrawing blood samples for gas analysis. The signal from the transducer was displayed on a pen-recorder (Devices, M4) and recorded on one channel of an FM tape recorder (Tandberg, 100; frequency response d.c. to 1250 Hz).

A femoral vein was cannulated and used for drug administration. The lingual artery on the same side as the sinus nerve from which recordings were obtained was cannulated with a catheter (o.d. 0·75 mm) with the tip positioned in the common carotid artery 2 cm caudal to the carotid bifurcation and this position was confirmed post mortem. In some experiments another catheter was similarly positioned in the contralateral common carotid artery.

Respiration. The lungs were artificially ventilated with room air by a respiratory pump (S.R.I.) operating at 18 or 25 (later experiments) rev/min. End-tidal CO_2 was continually monitored by an infra-red CO_2 analyser (med 1A; Grubb Parsons) and maintained at 5% by appropriate adjustment of the pump stroke volume. Anoxic stimulation was achieved by replacing air with 100% N_2 as the ventilating gas and allowing the animal to inhale this for 2 min before returning to air.

Blood from a femoral artery was withdrawn approximately every hour during the

experiment and P_{a,CO_2}, P_{a,O_2} and pH estimated using a Radiometer gas monitor (BMS3 with PHM71 meter). Plasma bicarbonate was maintained between 20 and 25 mM either by adjusting the stroke of the respiratory pump or by the I.V. injection of molar sodium bicarbonate solution, the base deficit being calculated from the nomogram of Singer & Hastings (1948). The bladder was drained at regular intervals and rectal temperature maintained at $38 \pm 0{\cdot}5$ °C.

Recording of sinus nerve activity. The sinus nerve was identified and sectioned central to its junction with the glossopharyngeal nerve. Exposed tissues were covered with warm (37 °C) mineral oil. Electrical activity from single or multiple chemoreceptor units was recorded from filaments of the peripheral nerve using bipolar platinum-iridium electrodes. Sensory nerve discharges were amplified by an a.c. amplifier (Neurolog, Digitimer), displayed on an oscilloscope (Tektronix, 5103N) and recorded on one channel of the tape recorder.

Chemoreceptor units were identified by their random discharge (Eyzaguirre & Lewin, 1961; Biscoe & Taylor, 1963) and their increase in discharge frequency following injection of 5 μg NaCN into the ipsilateral common carotid artery. The ganglioglomerular nerves (one to three sympathetic nerves from the superior cervical ganglion to the carotid sinus region (Eyzaguirre & Lewin, 1961)) were cut as were nerves which were found to course between the carotid sinus and the nodose ganglion in some animals. The ganglioglomerular nerves were cut in order to eliminate reflex effects of sympathetic activity on carotid nerve discharge (Floyd & Neil, 1952; Eyzaguirre & Lewin, 1961). Results obtained from this preparation (Fig. 1) agree with reports that chemoreceptor discharge is largely independent of blood pressure, at least over the physiological range (Hornbein, Griffo & Roos, 1961; Biscoe, Purves & Sampson, 1970; Acker, Keller, Lübbers, Bingmann, Schulze & Caspers, 1973).

Single units were identified from the constant shape and amplitude of the action potential. In most of the multi-unit recordings it was found that a high dose of stimulant evoked a maximum discharge ($X_{max.}$ c.p.s.) which remained fairly constant during the course of an experiment. If a unit was recruited, or another ceased responding, $X_{max.}$ changed and this provided a quick method for monitoring the units and was used in conjunction with visual checking of the action potentials on the oscilloscope. Analysis was performed only if the number of units being examined, usually 2–3, remained constant throughout the experiment.

Data analysis. The output of the tape channel containing the action potentials was fed to a pulse height discriminator, the upper and lower levels at which the discriminator operated being indicated by Z axis modulation. The analogue output, which had been stored for 1 sec, was fed to a digital voltmeter (Schlumberger, A210) coupled to a data transfer unit (Schlumberger, 3240) which drove an Addo 5 punch.

Average discharge ($\bar{x}$) in the pre-stimulus or 'control' period, generally 20 sec, was computed (PDP-8 computer, Digital Equipment Corporation) from the punched tape. The average and total counts (Σx) were calculated for each response after its duration (t sec) had been determined from a histogram of the response, displayed by the computer on an x–y plotter (Complot, Houston Instruments). Responses were expressed as increments above the control level by subtracting the appropriate values, i.e.:

$$\Delta\bar{x} = \bar{x}\ (\text{response}) - \bar{x}\ (\text{control}),$$

$$\Delta\Sigma x = \Sigma x\ (\text{response}) - \Sigma x\ (\text{control}),$$

where Σx (control) $= \bar{x}$ (control) $\times t$ (response duration, sec).

A 'response' was defined as being from the first substantial (i.e. 3 times or more) increase above the mean control discharge frequency until the discharge returned to the pre-injection level. Beidler (1954) expressed chemosensory receptor activity

in terms of the integrated neural response (Σx) and the same approach has been advocated for arterial chemoreceptor studies by Paintal (1971) because Σx allows for differences in response duration. Data were therefore expressed in terms of $\Delta\Sigma x$, and also $\Delta\bar{x}$ because previous workers have generally used the averaged discharge when evaluating drug effects. Expressing the drug results in terms of both variables made it possible to determine whether there was an appreciable difference in the information they provided (see also McQueen, 1974).

Dose–response data. $\Delta\bar{x}$ and $\Delta\Sigma x$ were plotted against the $\log_{10}$ dose of the stimulant. A straight line was fitted to the points in the linear portion of the dose–response curve using the method of least squares. The slope (m) and intercept (c) were calculated for each line and from the equation for a straight line, $y = mx + c$, it was possible to calculate the response (y) elicited by a dose of stimulant (x).

A response in the central region of the control or pre-drug dose–response line was selected arbitrarily and the dose of stimulant required to match this response following drug administration was calculated from the post-drug dose–response line. The ratio of the dose required after drug administration to that required in the control state is the *dose ratio* and this provided an objective assessment of a drug's influence on responses evoked by chemoreceptor stimulants. Dose ratios obtained from different experiments were pooled and data presented as the mean ratio ± S.E. of mean. The S.E. of mean is given to provide an estimate of the scatter of individual ratios about the mean value, although they are not necessarily normally distributed. Data for each drug were obtained from several animals in order to provide an estimate of the population response.

Gallamine. The animals were paralysed during the experiment with gallamine (3 mg/kg I.V.), the dose being repeated as required, usually every 1–1·5 hr. This neuromuscular blocking drug was given to prevent muscle contractions, either spontaneous or caused by the close-arterial injections of ACh, from moving the nerve on the recording electrodes, and also to suppress spontaneous respiratory movements which are associated with fluctuations in end-tidal CO_2 and blood pressure.

Chemoreceptor discharge frequency obtained with the animal artificially ventilated and paralysed (end-tidal CO_2 5%, P_{a,CO_2} about 34 mmHg) was very similar to that observed when it was breathing spontaneously, and remained relatively constant throughout the experiment – providing that the sympathetic nerve supply to the carotid body was cut and that mean blood pressure did not fall below 50 mmHg (see Fig. 3). Further, it was established that this dose of gallamine did not appreciably affect the response of the chemoreceptors to either ACh or NaCN (see Fig. 5).

Chemoreceptor stimulation. The effect of a chemoreceptor stimulant was determined by injecting 0·1 ml. of the solution into the common carotid artery via the lingual catheter and washing it in with 0·2 ml. Locke solution. Injections were made over 2 sec commencing at the peak of the inspiratory phase of the respiratory cycle and were repeated every 5 min. Other drugs were injected I.V. or intra-carotid over 5–20 sec and 10 min allowed before retesting the stimulants. The dead-space in the catheter was 0·1 ml. and it was flushed with 0·2 ml. Locke solution between injections.

Drugs. Drugs were prepared in modified Locke solution (NaCl 6·0 g; KCl 0·42 g; $CaCl_2$ 0·24 g; Tris base 6·0 g; N-HCl 39 ml.; distilled water to 1 l.; pH 7·41 at 37 °C). Doses referred to are those of the salts.

The drugs used in this investigation were: pentobarbitone sodium (Abbott Laboratories), gallamine triethiodide (May & Baker), acetylcholine iodide, sodium cyanide, atropine sulphate, physostigmine salicylate, carbamylcholine chloride (carbachol) (all B.D.H.); hemicholinium-3 (Aldrich), mecamylamine HCl (M.S.D.), α-bungarotoxin and β-bungarotoxin (Miami Serpentarium Laboratories, Miami, Florida, U.S.A.).

RESULTS

Experiments were performed on forty-three cats from which a total of sixty-nine recordings (seventeen single and fifty-two multiple units) of chemoreceptor activity were obtained.

Chemoreceptor responses to ACh and NaCN

In *all* the recordings ACh and NaCN were effective chemoreceptor stimulants. The threshold dose for stimulation varied slightly from recording to recording, generally being about 0·5 μg NaCN or 5 μg ACh. Maximum responses were evoked by 25–50 μg NaCN or 125–250 μg ACh, again with variation from one recording to another. NaCN elicited responses which had a longer latency to onset and lasted longer than comparable responses ($\Delta\bar{x}$) to ACh.

Vascular effects

The experiment shown in Fig. 1 illustrates that chemoreceptor activity in this preparation was largely independent of blood pressure. A large dose of ACh injected into the contralateral carotid artery caused a fall in blood pressure of the same magnitude as that seen following ipsilateral administration of the same dose, but without the chemoreceptor stimulation.

Since the chemoreceptor stimulants were not confined to the sinus region it was necessary to determine what effects they might have on recirculation. Experiments were performed on eight cats in which maximal or near-maximal doses of stimulant were injected into the contralateral carotid artery and the effect on chemoreceptor activity in the ipsilateral sinus nerve examined. It was found that these doses, which were higher than those used in dose–response studies, had little or no effect on the ipsilateral carotid body chemoreceptors, as can be seen from Fig. 1.

Analysis of chemoreceptor responses

It had been established by previous work (Diamond, 1955; McQueen, 1974) that there is a linear relationship between the $\log_{10}$ dose of stimulant and the chemoreceptor response over part of the dose–response curve. This was confirmed during preliminary experiments in the present investigation. However, the responses were rather scattered and fitting a dose–response line to the data by eye was subjective and, therefore, unsatisfactory. The relationship seemed to be sigmoidal, but the error involved in making the standard pharmacological assumption that response is linearly related to log dose over the central portion of the curve is

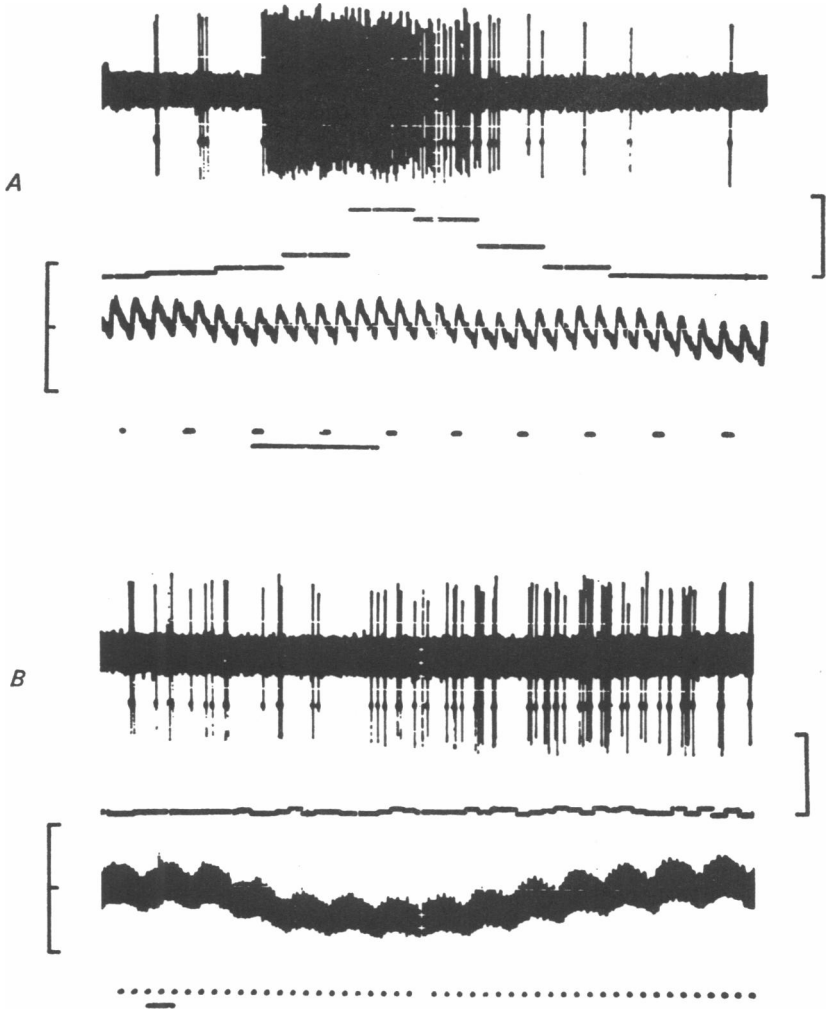

Fig. 1. Chemoreceptor unit from an experiment in which the influence of a high dose of stimulant on chemoreceptor activity in the contralateral sinus nerve was examined. *A*, ipsilateral injection of 125 μg ACh. *B*, the same dose administered on the contralateral side. Average spontaneous discharge was 0·8 c.p.s. before and 1·6 c.p.s. during the 20–30 sec period after injection of ACh. It can also be seen that hypotension did not appreciably affect chemoreceptor activity. Panels show from above downwards: nerve action potentials, the lower level at which the pulse height discriminator was operating being indicated by the brightening pulses; counter output in counts/sec, calibration on right of panel: 50 c.p.s.; B.P., calibration on left of panel: 0–100–200 mmHg; 1 sec time marker; injection marker.

likely to be negligible. Further experiments showed that dose–response curves obtained from one or two units were qualitatively similar to those obtained from multi-unit recordings (see Fig. 2).

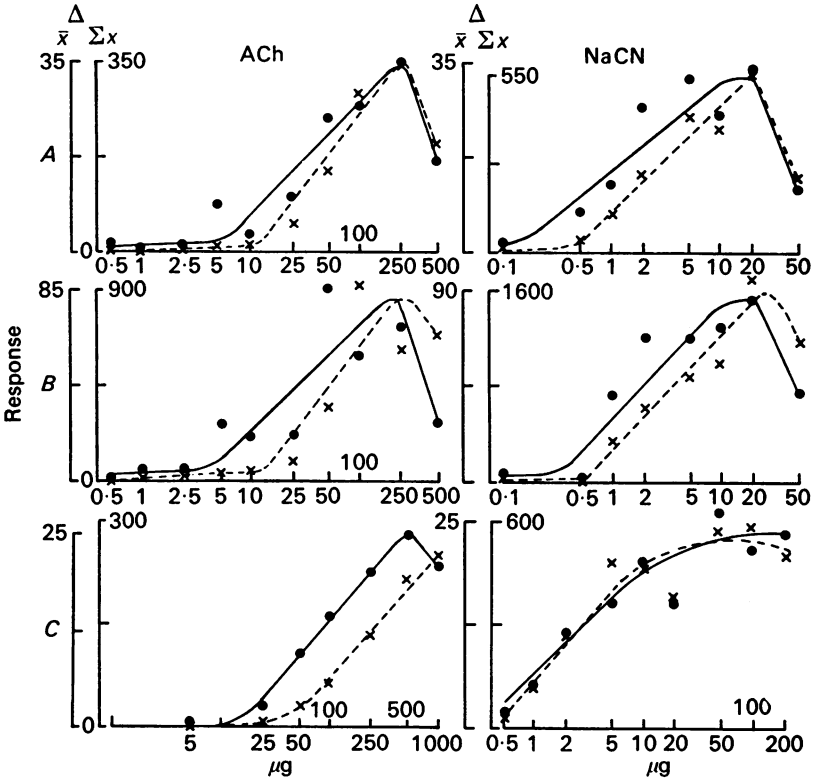

Fig. 2. Dose–response data obtained from two experiments. *A* and *B* are from the same recording, *A* being data from 2 units, *B* from 5 units. *C* is a single chemoreceptor unit obtained in a different experiment. Responses are plotted in this and subsequent figures ($\log_{10}$ scale) as $\Delta\bar{x}$ (●—●) and $\Delta\Sigma x$ (×---×) against the dose of either ACh or NaCN and the lines fitted to the points by eye.

To determine the constancy of the preparation's responsiveness during the long experiments required for dose–response studies, doses of stimulants were administered at regular intervals over several hours in three animals. Similar results were obtained, data from one of the experiments being shown in Fig. 3. These data provided an estimate of the variation in response to ACh and NaCN that might be expected during the long periods required for dose–response studies since other variables had been controlled as far as was possible and ACh, NaCN, gallamine and pentobarbitone had been administered, procedures which were to be common to

all other experiments. A variation of this magnitude in the dose–response ratio had to be accepted as inherent. The response to NaCN changed during the experiment, there being more counts elicited as the experiment progressed such that the $\Delta\Sigma x$ dose ratio decreased. However, the increased counts occurred over a longer time so that the $\Delta\bar{x}$ dose ratio was not affected.

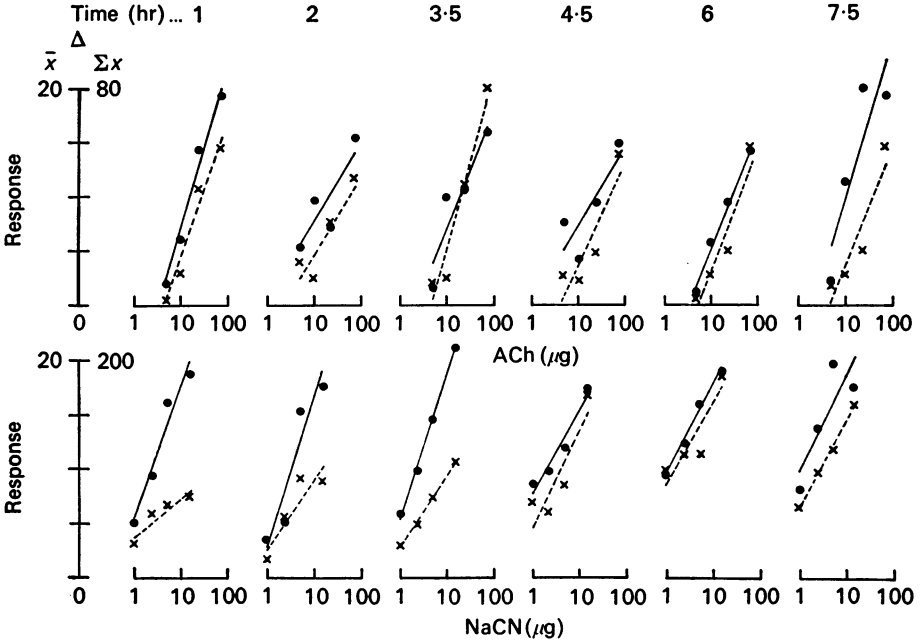

Fig. 3. Dose–response data from a single unit showing the effects of repeated doses of ACh and NaCN. Four doses of ACh and four of NaCN were injected in random order over a 45 min period, the sequence being performed six times during the course of a 9 hr recording. Gallamine was administered after each completed dose–response sequence - it was not present during the first cycle. Data are plotted as $\Delta\bar{x}$ (●—●) and $\Delta\Sigma x$ (×- - -×) against the dose of ACh or NaCN and the straight lines fitted by the method of least squares. The over-all mean spontaneous discharge, determined from the 20 sec control periods prior to injecting stimulants, was $2{\cdot}6 \pm 0{\cdot}2$ c.p.s., the average for individual sequences being in the range 1·8–3·4 c.p.s. The mean dose ratio (comparison against the first dose–response line) for ACh (20 μg) was $1{\cdot}4 \pm 0{\cdot}2$ ($\Delta\bar{x}$) and $1{\cdot}2 \pm 0{\cdot}1$ ($\Delta\Sigma x$). The corresponding ratios for NaCN (4 μg) were $1{\cdot}0 \pm 0{\cdot}1$ and $0{\cdot}6 \pm 0{\cdot}1$.

Drug effects

Drugs affecting the cholinergic system were used in this study and included antagonists such as mecamylamine, atropine and α-bungarotoxin, an anticholinesterase, and agents which interfere with the synthesis (HC-3) or release (β-bungarotoxin) of ACh. The results have been summarized in Fig. 5.

Mecamylamine. Mecamylamine itself caused inhibition of spontaneous activity lasting 10–15 sec which was followed by a slight increase in the discharge frequency, activity returning to control levels after about 30 sec. The lowest dose investigated was 0·1 mg/kg I.A. which gave dose ratios of 10 ($\Delta\bar{x}$) and 4·2 ($\Delta\Sigma x$) for ACh and 0·6 ($\Delta\bar{x}$) and 0·5 ($\Delta\Sigma x$) for NaCN, the reduction in response to ACh lasting for about 1·5 hr. In the remaining experiments mecamylamine was used at a dose of 1 mg/kg I.A. with, in some experiments, an additional 5 mg/kg I.A. administered later in the experiment.

Responses to ACh were blocked or substantially reduced by 1 mg/kg, it being impossible to determine the dose ratio since massive doses of ACh would have been required to overcome the inhibition and such doses (greater than 5 mg ACh I.A.) were not used since they would have caused substantial secondary effects. The ratio was therefore expressed as being > 10.

Whilst the response to ACh remained depressed for several hours, that to NaCN was not appreciably affected by mecamylamine (see Fig. 5). Even extremely high doses (up to 5 mg/kg I.A.), in excess of those reported by Nishi & Eyzaguirre (1971) as being capable of inhibiting the response to NaCN, had very little effect on the response to NaCN (see Fig. 4) even though tested over several hours.

A final attempt was made to inhibit the response to NaCN by injecting atropine (2 mg/kg) into the carotid artery about 1 hr after mecamylamine (5 mg/kg) had been given I.A. Nishi & Eyzaguirre (1971) described how in those chemoreceptor units in which the response to NaCN was not blocked by hexamethonium it was always depressed by injecting atropine (4 mg I.A., total dose). However, the results from the present experiments (see Figs. 4 and 5) showed only a slight inhibition of the cyanide effect, the response to ACh remaining inhibited by mecamylamine, and that to anoxic stimulation also being unaffected by the combination of mecamylamine and atropine.

The results show that mecamylamine, while reducing or abolishing the stimulation action of ACh on carotid chemoreceptor activity, had little or no effect on the response of these receptors to NaCN or anoxia.

Bungarotoxins. The response of chemoreceptors to ACh was reduced, although somewhat variably, by *α-bungarotoxin* (0·25 mg/kg I.A.). The reduction was not as great as that observed following mecamylamine (0·1 mg/kg I.A.). The response to NaCN was slightly inhibited, again with some variation from one experiment to another.

Responses to ACh and NaCN were examined before and after administering *β-bungarotoxin* (0·5 mg/kg). When the toxin had been present for 3 hr an additional dose of 0·5 mg/kg was injected I.A., and the animal was then subjected to anoxia for three 2 min periods over the course of

20 min. Nitrogen breathing evoked substantial and sustained increases in chemoreceptor activity. During the following 4 hr the anoxic stimulus was repeated from time to time, as were the chemical stimuli. The chemoreceptors were stimulated strongly over the course of several hours because at the neuromuscular junction it has been found that block of

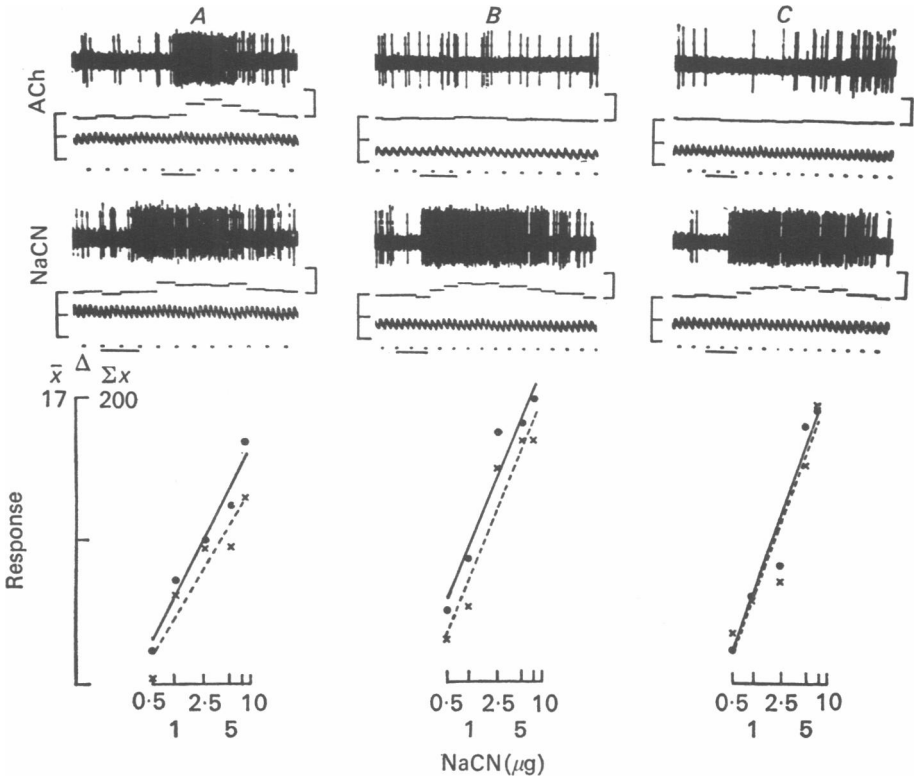

Fig. 4. Single chemoreceptor unit from an experiment in which the influence of high doses of mecamylamine and atropine on responses to ACh and NaCN was investigated. *A* shows the control response to ACh 250 μg and NaCN 5 μg, *B* the response to the same doses following mecamylamine (5 mg/kg I.A.) and *C* the response after the addition of atropine, 2 mg/kg I.A. Record details as for Fig. 1. The lower part of the figure shows the dose–response lines for NaCN, the details being the same as in Fig. 3.

transmission takes some time to occur and develops faster if the preparation is stimulated at a fairly high frequency (Chang, Chen & Lee, 1973).

The results obtained showed little or no change in the response of the receptors to either ACh or NaCN, and although there may be a slight inhibition of the ACh response, this was not intensified by the additional dose of β-bungarotoxin (see Fig. 5).

Hemicholinium-3 (HC-3). The effects of doses of 2 mg/kg I.V. (similar to that used by Eyzaguirre & Nishi, 1974) and 2 mg/kg I.A. were studied. Following administration of HC-3 the animals were made anoxic for three 2 min periods over the course of 20 min before testing the effects of ACh and NaCN. The responses to ACh were not obviously affected, while those to NaCN were found to be potentiated. Repeated anoxic stimuli did not alter either the response to anoxia or that to NaCN, even when continued for up to 5 hr after the I.A. dose of HC-3.

Additional data were obtained from four experiments in which a higher dose of 4 mg/kg was given I.A. In two of these animals α-bungarotoxin had previously been administered. Mean dose ratios of >10 ($\Delta\bar{x}$) and $1{\cdot}8 \pm 1{\cdot}1$ ($\Delta\Sigma x$) for ACh and $0{\cdot}1 \pm 0{\cdot}05$ ($\Delta\bar{x}$) and $0{\cdot}5 \pm 0{\cdot}1$($\Delta\Sigma x$) for NaCN were obtained. However, at this dose the blood pressure was very low and spontaneous chemoreceptor activity had increased such that it became difficult to compare responses before and after HC-3 and the data were not included in the results shown in Fig. 5. This inhibition of the ACh response may well have been due to a blocking action of high doses of HC-3 at the cholinergic receptor site (Martin & Orkand, 1961).

The results indicate that neither the response to NaCN or that to anoxia is depressed by HC-3, and indeed the response to NaCN is augmented.

Atropine. The effect of 1 mg/kg I.V. was investigated because it was intended to use this dose in experiments involving physostigmine – atropine would prevent excessive muscarinic actions, particularly hypotension, following ACh administration. Experiments were performed in which the 1 mg/kg I.V. dose was given and dose ratios determined for ACh and NaCN. Then an additional dose of 1 mg/kg was administered, this time close-arterial to the carotid body, and the dose ratios determined again. The results obtained (see Fig. 5) were rather variable, the overall pattern being a slight depression of the ACh response and no change or slight inhibition of the NaCN response.

Physostigmine (*Eserine*). The influence of physostigmine on the responses to ACh and NaCN was examined in atropinized cats (1 mg/kg I.V.). An initial dose of 0·2 mg/kg potentiated the response to ACh but not that to NaCN (see Fig. 5). An additional dose of 1 mg/kg I.A. further augmented the response to ACh, while that to NaCN remained more or less unaltered (see Fig. 5). The response to anoxia was not obviously affected by physostigmine, nor was the spontaneous discharge altered.

During three experiments the effect of carbachol, a cholinergic antagonist not destroyed by cholinesterase, was determined before and after physostigmine. The mean dose ratios obtained were $1{\cdot}0 \pm 0{\cdot}05$ ($\Delta\bar{x}$) and $1{\cdot}2 \pm 0{\cdot}2$ ($\Delta\Sigma x$) for the 0·2 mg, and $1{\cdot}7 \pm 0{\cdot}6$ ($\Delta\bar{x}$) and $1{\cdot}9 \pm 0{\cdot}4$ ($\Delta\Sigma x$) for

the 1 mg/kg dose of physostigmine. This implies that physostigmine potentiated ACh by inactivating cholinesterase and not by some non-specific action.

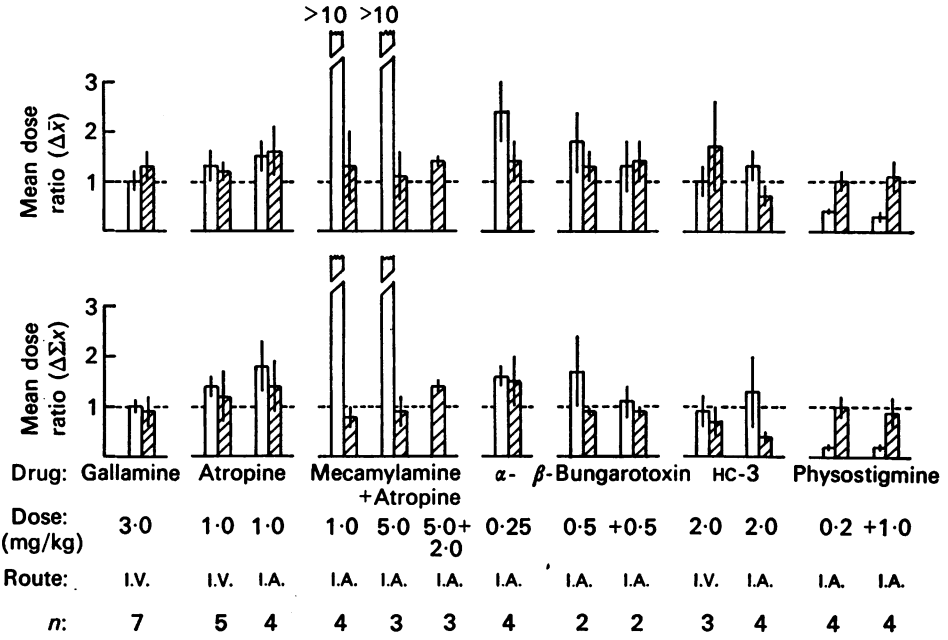

Fig. 5. Summary of the dose ratio data. The upper histogram gives the dose ratios ± S.E. of mean based on $\Delta\bar{x}$ and the lower histogram the ratios based on $\Delta\Sigma x$. Open rectangles are the ratios for ACh, the shaded rectangles the ratios for NaCN. The dashed line indicates the dose ratio of 1.

DISCUSSION

This study shows that drugs which affect the response of carotid chemoreceptors to ACh have little or no effect on the sensitivity of these receptors to NaCN or anoxia. The results are derived from a quantitative pharmacological investigation of drug effects on chemoreceptor activity and should be more reliable than those based on qualitative or semi-quantitative data. The extent to which this view is justified will be considered before discussing individual drug results.

Evaluation of drug effects

Electrical activity recorded from chemoreceptor units provides a more reliable indicator of chemosensory activity than do reflex changes, such as studied by Schweitzer & Wright (1938) and Byck (1961), because there are fewer secondary factors liable to influence the primary response and subtle changes can be detected. Single units were recorded in the present

investigation as were multiple units. There was no appreciable difference in the dose ratio data obtained by selectively counting single and multiple units (up to 6) in the same recording. This implies that the response of the population recorded is homogenous. Paintal (1967) found a difference between fast- and slow-conducting aortic chemoreceptor fibres in their responsiveness to ACh, the fast fibres being much less sensitive. The situation seems to be different in the sinus nerve where both types of fibre are sensitive to ACh and NaCN, the fast-conducting being slightly more sensitive (Sato, Fidone & Eyzaguirre, 1968; Fidone & Sato, 1969).

Chemoreceptor stimulants evoke variable responses and this presents problems when evaluating drug effects. If the variability resulted from secondary changes it should have been possible to minimize it by controlling these variables. However, even when secondary influences were controlled as far as was possible, some variation in response still occurred (see Fig. 3). The majority of authors who have performed drug studies on chemoreceptors support their conclusions by illustrating the response elicited by a particular dose of stimulant before and after administration of the drug being examined. The conclusions resulting from such single-dose studies may well be appropriate, but in view of the variability of chemoreceptor responses it would seem essential to provide quantitative data as supporting evidence rather than to show 'typical' responses. Dose ratios provide an objective estimate of a drug's effect on chemoreceptor activity.

Drug effects

Both ACh and NaCN were studied because they are classical stimulants around which much of the controversy concerning the ability of cholinergic drugs to influence chemoreceptor activity has revolved. Anoxia was also used and it provides a more physiological stimulus; the receptor's sensitivity to anoxia under the conditions in these experiments is akin to its sensitivity to NaCN. However, the response it evoked is gradual in onset with a long time course of action and is difficult to compare directly with the intense short-lasting responses evoked by ACh or NaCN.

Nicotinic antagonists

Probably the most controversial aspect of chemoreceptor pharmacology has been the conflicting evidence obtained from experiments involving ganglion blocking drugs. Some authors found that responses to ACh and NaCN (or hypoxia) were depressed by these agents (Landgren, Liljestrand & Zotterman, 1952; Joels & Neil, 1962; Nishi & Eyzaguirre, 1971; Eyzaguirre & Nishi, 1974) while others reported that although the response to ACh was depressed, that to NaCN or hypoxia was not (Moe, Capo &

Peralta, 1948; Douglas, 1952; Dontas & Nickerson, 1956; Anichkov & Belen'kii, 1963; Sampson, 1971). Tetraethylammonium (TEA) and hexamethonium were employed in the earlier studies while mecamylamine, which more readily penetrates membranes (Eyzaguirre & Zapata, 1968*b*), was used in the present experiments.

Mecamylamine completely inhibited the response to ACh without having much effect on the response to NaCN or anoxia even when high doses were used and several hours allowed for the antagonist to act (see Fig. 4). This finding is in agreement with the reports cited above that the chemoreceptor response to NaCN is not appreciably affected by ganglion blocking drugs. The argument of Moe *et al.* (1948) and Nishi & Eyzaguirre (1971) that the cyanide response is not depressed because the ganglion blocking drugs fails to reach an effective concentration at some 'intrinsic' site is not supported by the present results, and has previously been refuted by Gray & Diamond (1957).

There is a period of 1–2 min following the I.A. injection of high concentrations of drugs such as mecamylamine and atropine when the chemoreceptors do not respond to ACh, NaCN or anoxia. Nishi & Eyzaguirre (1970) also noted this and although they established it is not due to local anaesthesia, it seems unlikely that this short-lasting inhibition is a specific antagonism of endogenous ACh. It is probably a non-specific consequence of administering high concentrations of salts close-arterial to the carotid body.

The available evidence suggested that the action of *α-bungarotoxin* on cholinergic nerves is confined to the motor nerve of skeletal muscle (Chang & Lee, 1963). However, it was considered worth investigating the action of this substance on the chemoreceptor cholinergic receptor because D-tubocurarine is effective at this site and α-bungarotoxin appears to act at the same site as D-tubocurarine at the neuromuscular junction (Simpson, 1974). α-Bungarotoxin inhibited the response to ACh to about the same extent as did atropine. The inhibition was slight when compared with that caused by mecamylamine, and further studies are needed to establish whether the toxin is active at the same site as mecamylamine, or whether it acts elsewhere to reduce the response to ACh and, to a lesser extent, that to NaCN.

Muscarinic antagonist

Although it is generally agreed that the cholinergic receptor site in the carotid body is nicotinic (see Anichkov & Belen'kii, 1963), there are reports that *atropine* is effective in reducing chemoreceptor sensitivity to ACh and NaCN (Liljestrand, 1951; Landgren *et al.* 1952; Eyzaguirre & Nishi, 1974). This could be taken to imply that part of the response to ACh is mediated by muscarinic receptors, although it is more likely to be

a consequence of local anaesthesia (Heymans, Delaunois, Martini & Janssen, 1953) or antagonism at a nicotinic receptor site (Nishi & Eyzaguirre, 1971; McQueen, 1974). The results obtained showed atropine slightly inhibited the response to ACh, and to a lesser extent, that to NaCN. It is not known whether the inhibition observed was due to effects on nicotinic receptors or to some other action of atropine.

HC-3 and β-bungarotoxin

HC-3 has been used by Eyzaguirre and co-workers (Eyzaguirre & Zapata, 1968*b*; Nishi & Eyzaguirre, 1971; Eyzaguirre & Nishi, 1974) to prevent the synthesis to ACh in the carotid body. They found that treatment with HC-3 followed by periods of hypoxia (to deplete the stores of ACh) resulted in a diminished response to NaCN without affecting the response to ACh. In the present experiments the response to NaCN was depressed, sometimes markedly, as shown by $\Delta\bar{x}$ dose ratio data (Fig. 5). At first sight this agrees with Nishi & Eyzaguirre's findings. However, if the $\Delta\Sigma x$ ratio is considered, it can be seen that the response was slightly potentiated. The explanation for the difference between ratios is that the integrated response was greater but spread over a longer time, so that although the $\Delta\Sigma x$ ratio decreased, the $\Delta\bar{x}$ ratio increased. Intra-arterial HC-3 further augmented the response to NaCN, both variables giving similar dose ratios.

The potentiation of the NaCN response by HC-3, and also by low doses of mecamylamine, could mean that the cyanide response is normally partially suppressed by a cholinergic mechanism. This is, however, only speculation since the cause of the potentiation following administration of these drugs has not been determined.

β-Bungarotoxin acts at the neuromuscular junction presynaptically and prevents the release of ACh (Chang *et al.* 1973). If it acts in a similar way at the chemoreceptor it would be expected that any stimulant which acts by releasing endogenous ACh would be rendered ineffective. The response to exogenous ACh should not be affected since there is no evidence of post-synaptic receptor blockade (Simpson, 1974). In the doses used in the chemoreceptor experiments β-bungarotoxin did not affect the cyanide response. The negative result could mean that activity evoked by NaCN is not dependent on ACh, but this would be the case only if β-bungarotoxin at the dose level studied can be shown to be effective in preventing the release of ACh in the carotid body. Further studies are required.

Anticholinesterases

The possibility that ACh might be involved in physiological transmission at chemoreceptors was raised by Schweitzer & Wright (1938) because of

the similar respiratory effects evoked by neostigmine and ACh. Others found the response to hypoxia was potentiated by physostigmine (Liljestrand, 1951; Landgren *et al.* 1952) whereas Heymans, Bouckaert and Pannier (1944) concluded from experiments in dogs that although the response to ACh was potentiated, that to other stimulants was not. Present results demonstrate that the response to exogenous ACh is potentiated by *physostigmine* while that to NaCN is definitely not influenced. Both acetylcholinesterase and pseudocholinesterase are located in the cat carotid body (Biscoe & Silver, 1966).

In conclusion, the increased chemoreceptor activity evoked by NaCN was not affected by either atropine, mecamylamine or physostigmine in the doses used, but was augmented by HC-3. Responses to ACh were quite clearly inhibited by mecamylamine and potentiated by physostigmine, this being in accord with the consensus in the literature. The thesis of this study is that conflicting evidence concerning the actions of cholinergic drugs on the chemoreceptors arises from subjective interpretation of qualitative data. Since conclusions reached in the present work are based on objective evidence obtained from quantitative pharmacological data, they should help to resolve the conflict of evidence referred to by Heymans & Neil (1958).

Cholinergic theory of chemosensory transmission

It should be appreciated that it was not the intention to investigate the mechanism whereby ACh, NaCN, or anoxia excite chemosensory nerves. However, the results obtained make it extremely unlikely that endogenous ACh is involved as an intermediary in the chemoreceptor response to NaCN and, therefore, do not support the theory that ACh is an excitatory sensory transmitter in the carotid body. Some of the most persuasive evidence in favour of the cholinergic theory has come from pharmacological studies on isolated carotid bodies *in vitro* (Eyzaguirre & Zapata, 1968*b*), but unfortunately direct comparison of this with evidence from the present experiments is not feasible because of the completely different experimental conditions.

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