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EFFECTS OF ACETYLCHOLINE AND SODIUM CYANIDE ON CAT CAROTID BARORECEPTORS

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1 The effects of intracarotid (i.a.) injections of acetylcholine (ACh) and sodium cyanide (NaCN) on baroreceptor activity recorded from the sinus nerve have been investigated in cats anaesthetized with pentobarbitone.

2 Two types of baroreceptor unit were recorded. The predominant type discharged at least 3 to 4 spikes per pulse wave at normal BP; they are referred to as 'polyspike' units and may have been associated with A fibres. The other type discharged a maximum of 1 to 3 spikes per pulse wave, even at high BP; they are referred to as 'few-spike' units and may have been from C fibres.

3 NaCN had no direct effect on either type of baroreceptor unit, even when injected in high doses (2.04 to 5.1 μmol i.a.) which cause maximal chemoreceptor stimulation, and it is concluded that as far as the cat's carotid baroreceptors and chemoreceptors are concerned, NaCN is a specific chemoreceptor stimulant.

4 ACh had no direct effect on polyspike baroreceptor units unless very high doses (1.83 μmol i.a.) were injected, when there was occasionally a transient slight increase in discharge. This effect appeared to be secondary to muscle contraction caused by ACh since it was not seen when an adequate neuromuscular-blocking dose of gallamine had been administered.

5 ACh stimulated the few-spike type of baroreceptor unit, an effect which was dose-related and lasted for up to 3 s; the threshold dose for baroreceptor stimulation was higher than that needed to excite chemoreceptor units. The increased discharge also occurred during experiments in which gallamine had been administered. Only five of these units were recorded during the investigation, despite an intensive search for them.

6 There was a delayed increase in baroreceptor sensitivity following the administration of ACh in doses (37 to 366 nmol i.a.) which had no immediate direct effect on polyspike baroreceptor discharge. The effect was evidently not secondary to changes in sympathetic nerve activity to the sinus region since it was observed during an experiment in which the ganglioglomerular nerves had been cut. Whether the increased sensitivity resulted from direct or indirect actions of ACh remains to be determined.

7 It is concluded that low doses of ACh or other drugs with nicotinic properties are unlikely to evoke baroreceptor reflexes on intracarotid injection, although they may cause delayed changes in baroreceptor sensitivity. Higher doses of ACh do not directly affect baroreceptor polyspike (A fibre) units, but transient baroreflex changes might result from stimulation of baroreceptor few-spike (C fibre) units. It is most unlikely that NaCN has any direct effect on baroreceptor reflex activity when injected into the carotid artery in doses used to elicit chemoreceptor reflexes.

Introduction

Sodium cyanide (NaCN) is a classical chemoreceptor stimulant (see Heymans & Neil, 1958). However, Fahim, Paintal & Torrance (1972) have found that some aortic chemoreceptors in cats were not excited by NaCN and some gastric stretch receptors were. They conclude 'that NaCN is definitely not a specific chemoreceptor stimulant'. Furthermore, Dontas (1954) claimed that NaCN exerted a stimulant action on the carotid baroreceptors and Paintal (1977) considered that 'it would not be surprising if sodium cyanide also stimulated the carotid baroreceptors with non-medullated fibres'.

These points raise the question of whether investigators who used NaCN during the study of chemoreceptor reflexes (e.g. see Heymans & Neil, 1958; McQueen, 1970) were evoking responses which were the result of mixed baro- and chemoreceptor stimulation. They also cause one to question whether the

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identity of a unit recorded from the sinus nerve can be based, reliably, on its responsiveness to NaCN. It was decided to perform an electrophysiological study of the effects of NaCN on carotid baroreceptors in the cat in order to try and answer these questions. Responses to acetylcholine (ACh) were also investigated because there is some confusion in the literature concerning the effect of this drug on the baroreceptors. Euler, Liljestrand & Zotterman (1941) found that ACh increased chemoreceptor discharge without affecting the 'great pressure spikes' in recordings of sinus nerve activity, and Landgren, Skouby & Zotterman (1953) considered that ACh was not an adequate stimulus for the baroreceptors, although it did alter their sensitivity to other stimuli. Diamond (1955) used an *in vitro* preparation of the carotid sinus and found that ACh stimulated the carotid baroreceptors.

Methods

Experimental animals

Cats of either sex weighing between 2.1 and 3.9 kg (median weight 2.9 kg, $n = 26$ cats) were used. They were anaesthetized with pentobarbitone sodium (42 mg/kg i.p.) supplemented approximately every 1 to 2 h during the experiment by intravenous administration of 10% of the initial dose. For some of the experiments the animals breathed spontaneously, but for the majority they were artificially ventilated with room air and usually paralysed by gallamine triethiodide (3 mg/kg i.v.). End-tidal CO_2 was continuously monitored by an infra-red CO_2 analyser (Med 1A; Grubb Parsons) and the Pa_{CO_2}, Pa_{O_2} and pH of femoral arterial blood samples measured at regular intervals.

General details

Blood pressure was recorded from one femoral artery and the other was cannulated for arterial blood sampling. Rectal temperature was maintained at 38 ± 0.5°C by a heating pad and the bladder was drained regularly.

Drug solutions (0.1 ml) were injected into the common carotid artery ipsilateral to the sinus nerve from which activity was being recorded and washed in with 0.2 ml modified Locke solution which had been bubbled with 5% CO_2:95% air in a water bath at 37°C. The catheter was introduced into the common carotid artery via the lingual artery and advanced until its tip lay about 2 cm caudal to the carotid bifurcation. Injections were made over a 2 s period.

Recording and analysis of sinus nerve activity

This has been fully described previously (McQueen, 1977; Docherty & McQueen, 1978) and only a brief summary follows. A carotid sinus nerve was dissected free from surrounding tissues, cut centrally, and the electrical activity of single or few unit baroreceptor units recorded from the peripheral nerve with bipolar platinum-iridium electrodes and an a.c. amplifier (Neurolog; Digitimer). Nerve activity was recorded on tape (Tandberg 115; d.c.-1250 Hz) and subsequently analysed with the aid of a computer (PDP-8; Digital Equipment Corporation) in order to provide data concerning discharge frequency (e.g. average discharge in ct/s); histograms were obtained from an x–y plotter (Complot, Houston Instruments).

Identification of baroreceptor units

Baroreceptors were identified by the synchrony between the bursts of nerve activity and the rise in pulse pressure. Occlusion of the common carotid artery caudal to the carotid bifurcation led to a reduction or abolition of the discharge which was immediately restored on removing the occluding artery clip. Probing the sinus region caused increased unit activity, whereas injection of 0.3 ml Locke solution saturated with CO_2 had no effect on the discharge, although it strongly stimulated the chemoreceptors. Individual units were identified from the constant shape and amplitude of the action potential.

Drugs

Drugs were prepared in modified Locke solution (McQueen & Eyzaguirre, 1974) and were: pentobarbitone sodium, gallamine triethiodide (May & Baker); acetylcholine iodide, mol. mass 273; sodium cyanide (BDH), mol. mass 49.

Results

Different types of baroreceptor discharge

The most common baroreceptor discharge, obtained in 30 of the 35 units recorded (i.e. 86%), was of the type illustrated in Figure 1, namely polyspike activity associated with the increase in pulse pressure, the number of spikes per beat being related to the mean BP. There was usually no discharge below 40 to 50 mmHg, but above this threshold baroreceptor discharge increased with increasing pressure until a maximum discharge was attained at about 200 mmHg. At physiological pressures (90 to 150 mmHg) there were always at least 3 spikes per beat, usually 5 to 15, from a single unit.

The other type of activity observed is illustrated in Figure 3. This was encountered in 5 of the 35 (14%) units studied and was characterized by having a maximum of 1 to 3 spikes per beat and a higher threshold

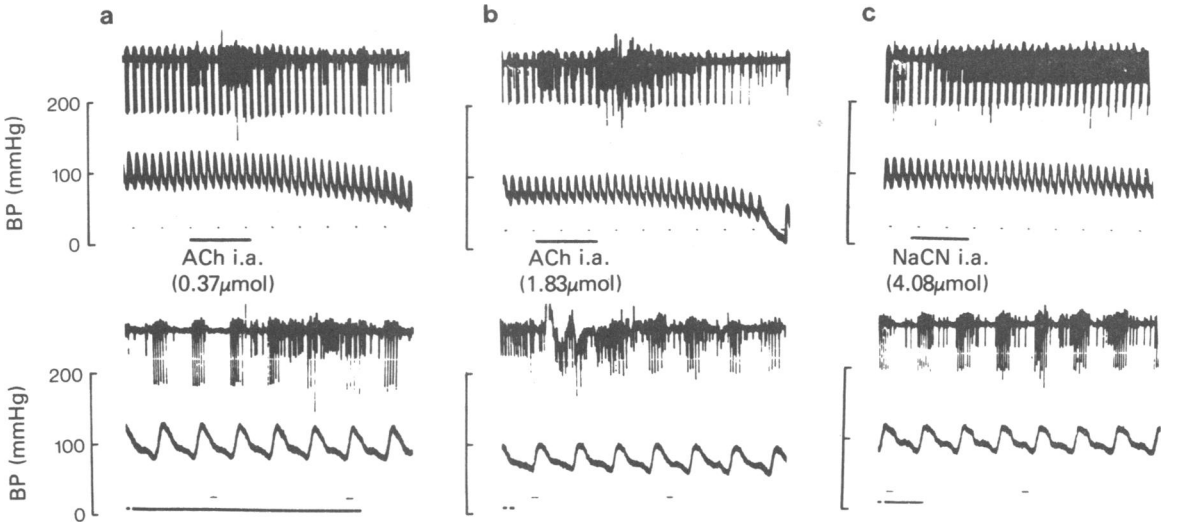

Figure 1 Effects of acetylcholine (ACh) and sodium cyanide (NaCN) on baro- and chemoreceptor activity in a non-paralysed spontaneously breathing cat with intact ganglioglomerular nerves: (a) shows the response to ACh (0.37 μmol i.a.), the lower panel being a faster oscilloscope sweep of the injection period. It can be seen that this dose of ACh markedly increased chemoreceptor discharge (smaller units) without affecting baroreceptor activity (larger unit). When the dose was increased to 1.83 μmol ACh, (b), muscle contraction occurred and the signal was distorted by movement of the nerve on the recording electrodes. A high dose of NaCN (4.08 μmol) (c) caused strong chemoreceptor excitation, but had no effect on baroreceptor activity. Panels show from above downwards: nerve action potentials; femoral BP; 1 s time marker; injection marker.

(mean BP, 70 to 100 mmHg). It was found that the duration of the action potential tended to be longer for the 'few-spike' group (1.5 to 4 ms) than it was for the 'polyspike' group (0.5 to 1.5 ms).

An assumption was made, and will be justified in the Discussion, that polyspikes are associated with fast-conducting (A) fibres in the sinus nerve, whereas the 'few-spike' type of activity is associated with slow-conducting (C) fibres.

Responsiveness of baroreceptor polyspike units (A fibres) to acetylcholine and sodium cyanide

ACh and NaCN were tested on 30 polyspike recordings obtained from 24 cats. Doses injected ranged from 3.7 nmol to 1.83 μmol (i.a.) for ACh and 20 nmol to 5.1 μmol (i.a.) for NaCN. The discharge pattern during the first 5 to 10 s following an injection was compared with that observed following a control injection of the same volume of Locke solution, which occasionally evoked a slight increase in discharge during the injection period. It was evident from early results that neither drug was having much effect on baroreceptor activity, so in subsequent experiments only the higher doses were studied.

Gallamine Experiments were performed on 5 cats which were not paralysed. It was found that higher doses of ACh ($\geq$0.18 μmol) caused a transient contraction of the muscles in the neck which often resulted in movement of the nerve on the recording electrodes and distortion or loss of the signal. There was no evidence of baroreceptor stimulation following high doses of ACh, although the situation during the period of muscle contraction was difficult to assess, nor did high doses of NaCN affect the discharge (see Figure 1).

In the other 19 cats, gallamine was administered to prevent muscle movements from affecting discharge. Neither ACh or NaCN in high doses had any stimulant action on the baroreceptor units in 16 of the experiments. However, in 2 cats ACh (1.83 μmol i.a.) did increase discharge during the injection (see Figure 2). This effect coincided with slight muscle contraction in the neck, indicating that there was insufficient gallamine present to prevent the high dose of ACh from contracting muscles and thereby transiently affecting discharge. Again, NaCN was without effect.

Sympathetic innervation of the carotid sinus The ganglioglomerular nerves (Floyd & Neil, 1952; Eyzaguirre & Lewin, 1961) were left intact in seven experiments and cut in the remainder. ACh and NaCN were without effect in all the experiments in which the sinus symapthetic innervation was intact. Five of the cats were paralysed with gallamine, two were not.

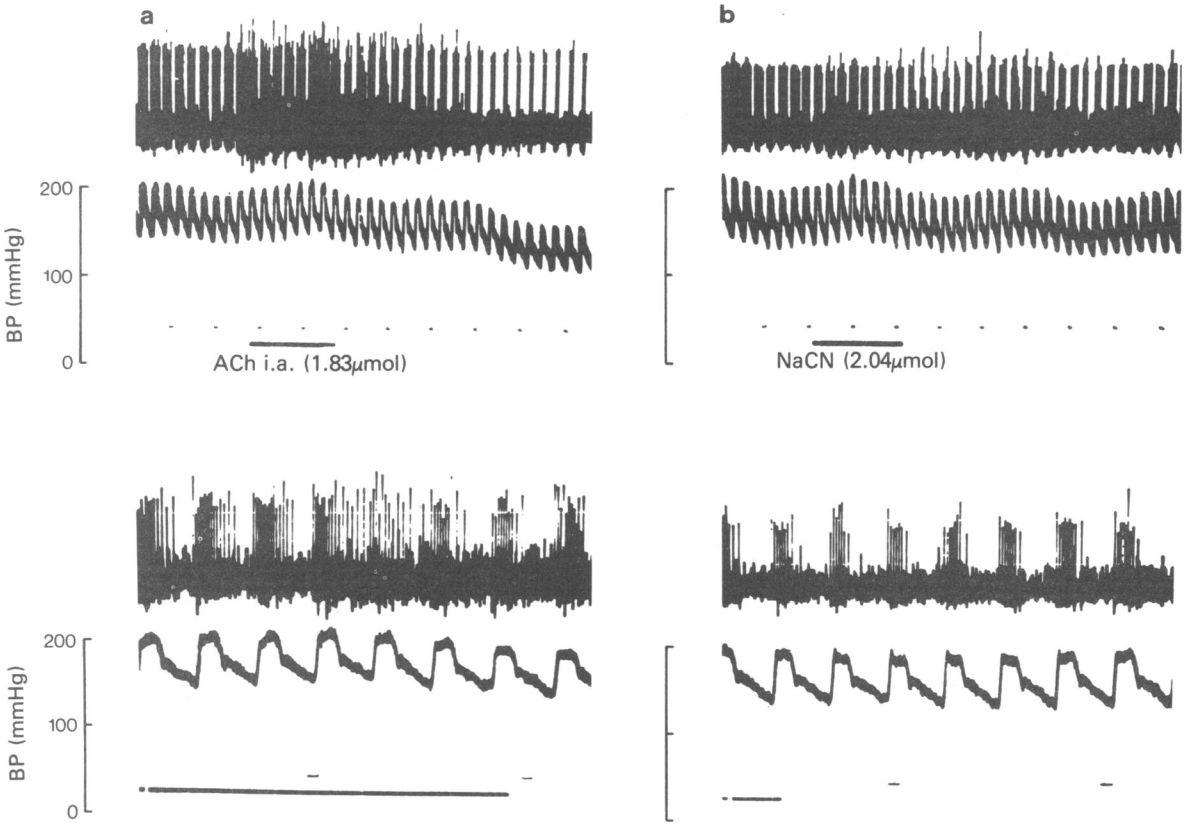

Figure 2 In this experiment the cat was artificially ventilated, paralysed by gallamine and the ganglioglomerular nerves were cut: (a) shows the effect of acetylcholine (ACh, 1.83 μmol i.a.), the lower panel being a faster sweep of the injection period; (b) is the response to sodium cyanide (NaCN, 2.04 μmol). Both drugs stimulated the small chemoreceptor units and ACh, but not NaCN, caused a transient excitation of the baroreceptors, an effect which coincided with muscle contraction in the neck. Record details as for Figure 1.

Responsiveness of baroreceptor few-spike units (C fibres) to acetylcholine and sodium cyanide

ACh and NaCN were tested on 5 few-spike recordings obtained from 5 cats in which the ganglioglomerular nerves had been cut. Two of the animals were paralysed with gallamine, the others were not. Doses of ACh (37 nmol to 0.92 μmol) and NaCN (0.1 to 2.04 μmol) were injected (i.a.) and it was found that whereas higher doses of ACh sometimes increased baroreceptor activity, NaCN was without effect. The biggest response to ACh was obtained from an experiment in which gallamine had been administered (see Figure 3). The lower dose of ACh had only a slight effect on discharge, but the high dose caused an intense discharge, unrelated to pulse pressure, which lasted for 3 s. A high dose of NaCN did not cause any increase in discharge.

Not all these units displayed the same sensitivity to ACh. Thus, the baroreceptor shown in Figure 4a was excited by ACh (0.18 μmol), the recording having been obtained from a gallamine-treated cat, whereas that shown in Figure 4b was unaffected by this dose of ACh. An attempt was made to determine whether the latter unit responded to 0.92 μmol ACh, but unfortunately the dose caused violent muscle contractions and the recording was lost, this being one of the hazards of injecting large amounts of ACh into non-paralysed animals. NaCN (1.02 μmol i.a.) had no effect on either unit.

Sensitisation of baroreceptors

The effect of various doses of ACh (37 nmol to 1.83 μmol) was studied on a single polyspike baroreceptor recorded from an artificially ventilated paralysed ani-

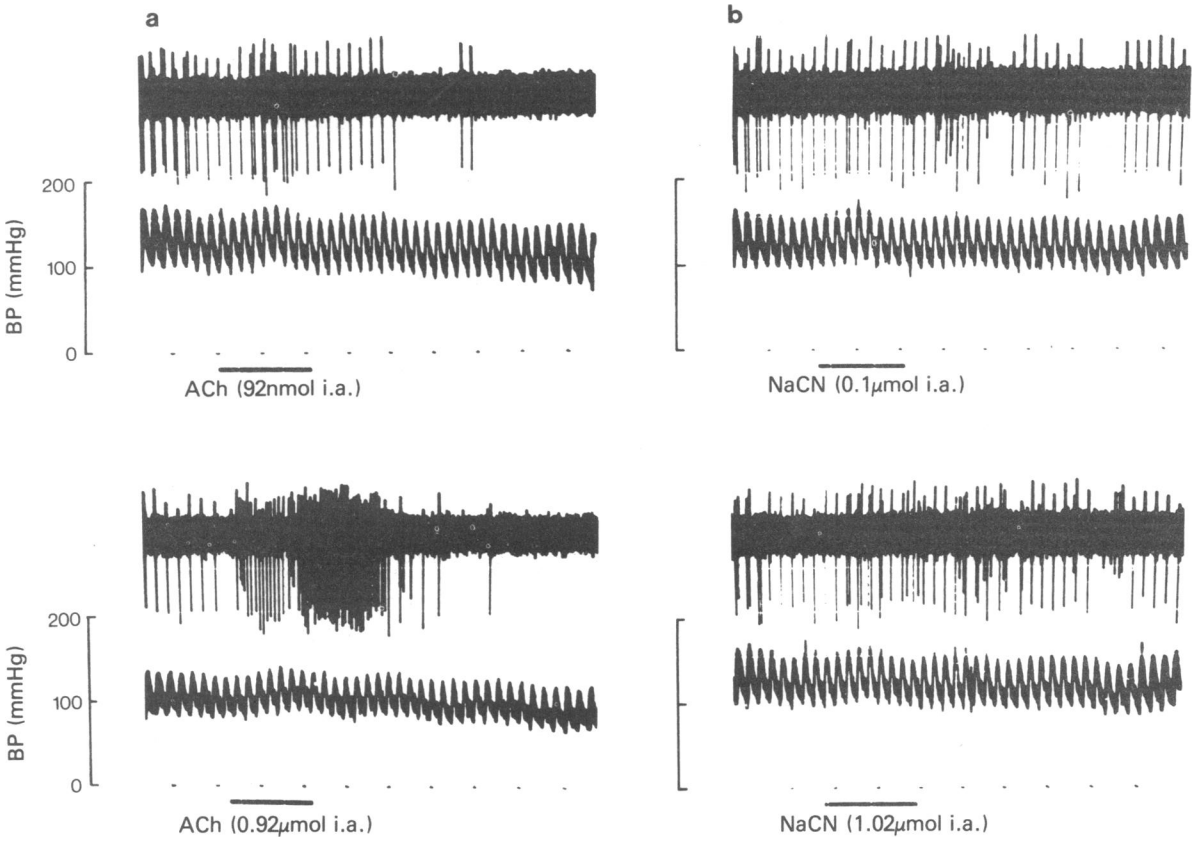

Figure 3 A recording of 'few-spike' baroreceptor activity from a paralysed cat in which the ganglioglomerular nerves were cut: (a) shows the responses to 92 nmol and, below, 0.92 μmol of acetylcholine (ACh). The low dose caused a slightly increased baroreceptor discharge and the high dose caused a marked increase in activity. In contrast, sodium cyanide (NaCN, 0.1 and, below, 1.02 μmol) had no effect on the discharge. Record details as for Figure 1.

mal in which the ganglioglomerular nerves were cut. It was noted that discharge was potentiated during the period following the hypotension evoked by lower doses of ACh, at a time when mean BP and pulse pressure were about the same as in the control period (see Figure 5). Discharge was not increased above control levels following higher doses of ACh, but mean BP remained below control levels during the first 90 s after the injections. Allowing for the lower BP, discharge was, in fact, potentiated. The increased discharge could not be accounted for entirely by the increase in heart rate, although this did make a small contribution.

Discussion

None of the baroreceptor units recorded was stimulated by NaCN, despite the use of high doses which cause maximal chemoreceptor excitation (McQueen, 1977). Dontas (1954) presented no evidence to support his assertion that NaCN (0.1 to 1 mg, i.a.) stimulates baroreceptors in cats and dogs, and Paintal (1977) was merely speculating when he suggested that cyanide might stimulate baroreceptors with non-medullated fibres. The lack of evidence in the literature to support the notion that NaCN stimulates baroreceptors, and the present failure to demonstrate any such effect, makes it reasonable to conclude that low doses of NaCN used to activate the chemoreceptors during the study of carotid chemoreceptor reflexes will not directly affect the baroreceptors.

In contrast to the findings of Fahim *et al.* (1972) on *aortic* chemoreceptors, none of the *carotid* chemoreceptor units obtained by the author from over 250 recordings in 168 cats has failed to respond to NaCN (0.1 μmol i.a.). It appears, therefore, that as far as the cat carotid baroreceptors and chemoreceptors are

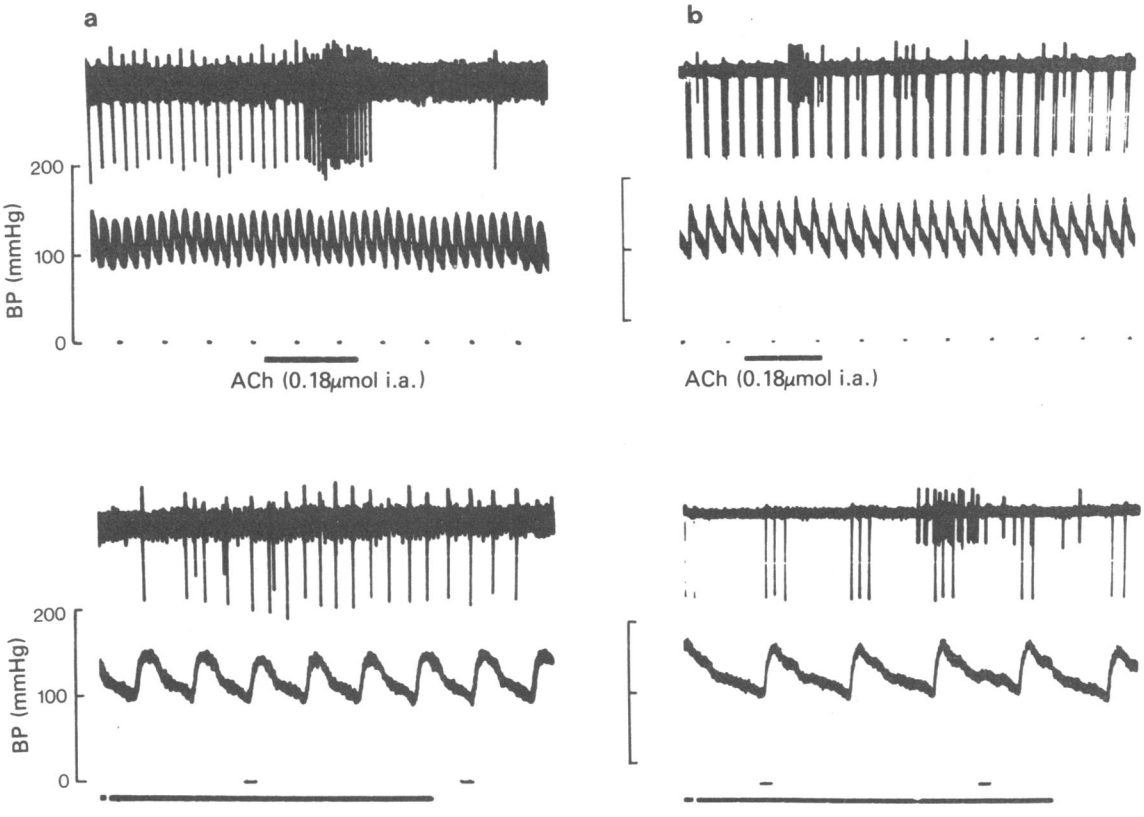

Figure 4 Responses to acetylcholine (ACh, 0.18 μmol) recorded from 'few-spike' recordings of baroreceptors in two different animals. The responses shown in (a) was from a paralysed cat; a fast sweep of the injection period is shown below the slow oscilloscope sweep. Discharge was increased in this experiment, whereas in (b) a recording from an unparalysed cat showed no baroreceptor response to the same dose of ACh, although chemoreceptor units were stimulated. Record details as for Figure 1.

concerned, NaCN is a specific chemoreceptor stimulant. With ACh the situation is more complicated. 'Polyspike' baroreceptor units were only affected by very high doses of ACh, and this effect was evidently secondary to muscle contraction since it was not seen when the neuromuscular blocking drug gallamine was administered in doses which have no effect on the response of the chemoreceptors to ACh (McQueen, 1977).

The 'few-spike' baroreceptor units, in contrast, tended to be excited by ACh, although the doses needed to do this were greater than those needed to stimulate chemoreceptors. The effect was not due to ganglionic stimulation by ACh of the sympathetic supply to the carotid sinus (Kezdi, 1954; Sampson & Mills, 1970; but cf. Floyd & Neil, 1952; Simón, Zamorano, Yajeya & Belmonte, 1976) because it was obtained during experiments in which the ganglioglomerular nerves had been cut. Neither was it secondary to muscle contraction, since the increase in baroreceptor discharge was observed in paralysed animals.

The only baroreceptor units to respond to ACh (<1.83 μmol i.a.) were of the few-spike type which had long-duration potentials, a feature which could be taken as evidence that they were associated with slow-conducting (C) fibres (Gasser, 1950; Paintal, 1966). It would have been desirable to measure their conduction velocity, but the short length of nerve available makes this technically very difficult (see Paintal, 1971). However, Fidone & Sato (1969) found that: 'baroreceptor C fibres seldom discharge more than 1–2 impulses per pulse wave, whereas baroreceptor A fibres commonly respond with 3–5 impulses or more'. They also found that ACh, in doses greater than those needed to excite chemoreceptor fibres, caused a slight stimulation of baroreceptor C fibres. Thus, the discharge pattern of the few-spike units,

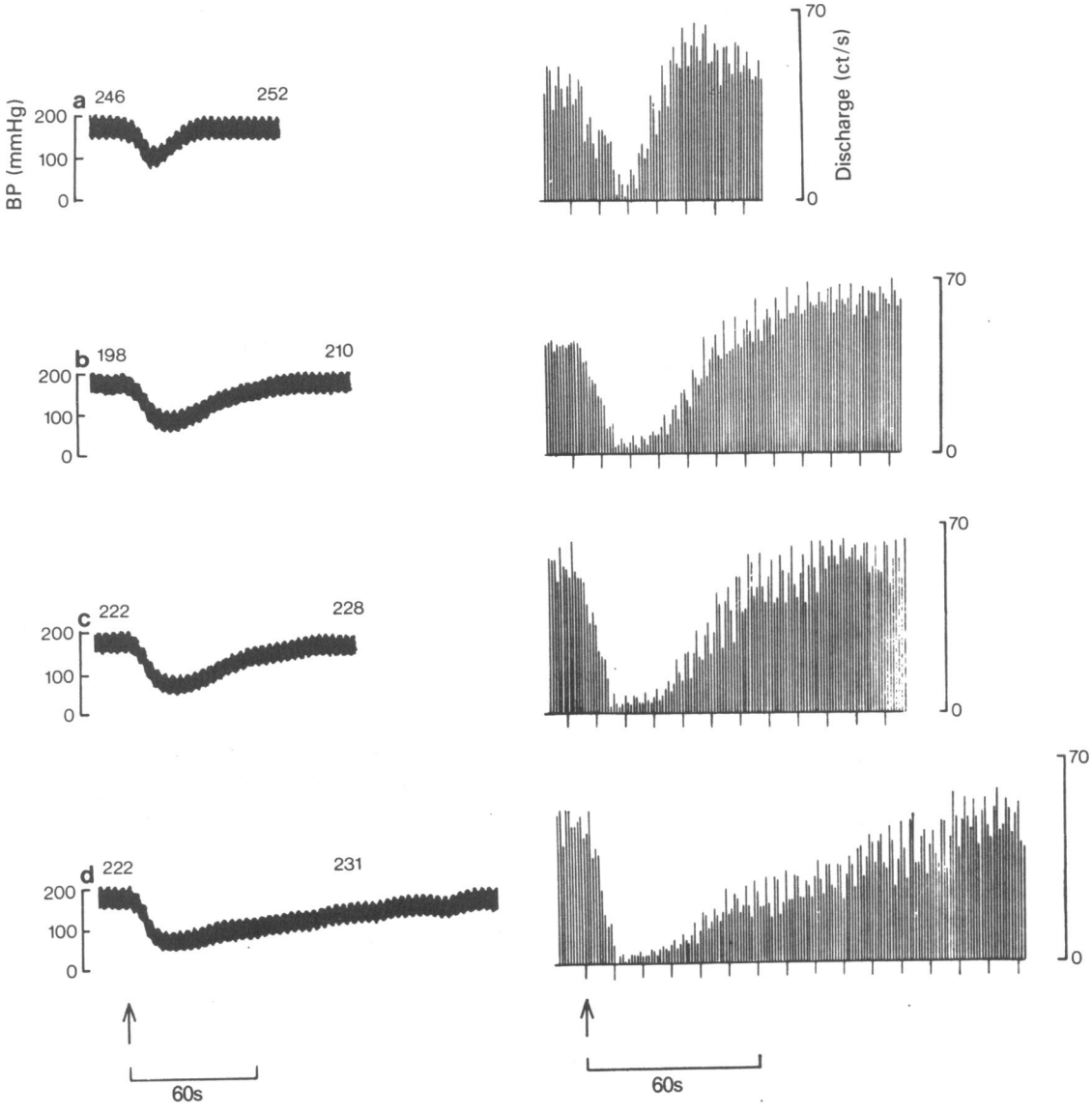

Figure 5 Data obtained from an experiment on an artificially ventilated paralysed cat with the ganglioglomerular nerves cut. Discharge of a single polyspike baroreceptor unit was counted and the computed discharge, plotted by the x–y recorder, is shown on the right of the figure, with the accompanying record of BP on the left. Injections of acetylcholine (ACh) in the following doses were made at the points represented by the arrows: (a) 37 nmol; (b) 0.37 μmol; (c) 0.92 μmol; (d) 1.83 μmol i.a. The average heart rate in the 10 s period immediately preceding the injection was determined, as was the rate during the period 45 to 54 s (a) or 90 to 99 s (b–d) post-injection and the values (beats/min) are given above the BP traces.

together with their responsiveness to ACh (a feature of many non-myelinated fibres, Armett & Ritchie, 1961) strongly suggests that they were baroreceptor C fibres.

The results obtained are not in conflict with the finding of Euler *et al.* (1941) that ACh (5 to 10 μg) has no effect on the great pressure spikes (probably A fibre baroreceptors). Diamond (1955) showed that ACh stimulates the cat carotid baroreceptors *in vitro*, but comparison of his results with those from the

present study is difficult because of the great difference in experimental conditions. He found that small spikes were more affected by ACh than were larger spikes; indeed, some of the latter were unaffected even by very high doses of ACh. This may be taken as indirect evidence that C fibres (small spikes) were more readily affected by ACh than were A fibres (Kirchheim, 1976; but see Iggo, 1958), although it is not clear what proportion of the small spikes were chemoreceptors.

Landgren *et al.* (1953) found that although ACh (10 to 100 μg, i.a.) does not stimulate the baroreceptors, it does increase the receptor sensitivity. Similar findings were made in the present study and further experiments are needed to determine whether this increased sensitivity results from direct or indirect actions of ACh; it is evidently not dependent on intact sympathetic innervation of the sinus because it was observed when the ganglioglomerular nerves had been cut.

In summary, *low doses* of ACh or other drugs with nicotinic properties are unlikely to evoke baroreceptor reflexes on intracarotid injection, although they may cause delayed changes in baroreceptor sensitivity. Higher doses of ACh do not directly affect baroreceptor A fibres, but transient baroreflex changes might result from stimulation of baroreceptor C fibres, although any such changes would probably be masked by the concomitant intense chemoreflex activity evoked by ACh.

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