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THE INFLUENCE OF THE SYMPATHETIC OUTFLOW ON AORTIC CHEMORECEPTORS OF THE CAT DURING HYPOXIA AND HYPERCAPNIA

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SUMMARY

1. An attempt has been made to reconcile differing observations, made by different groups of investigators, on the responses of aortic chemoreceptors of cats during normoxia, hypoxia and hypercapnia.

2. In cats anaesthetized with sodium pentobarbitone it was observed that during hypoxic stimulation of twelve chemoreceptors, an intravenous injection of about 20 mg sodium pentobarbitone produced hypotension which was accompanied by an initial fall in chemoreceptor activity instead of the expected increase that invariably occurred in all the receptors when hypotension was produced mechanically by distending a balloon in the right atrium (twenty-six during normoxia, eleven during hypoxia and eight during hypercapnia).

3. In twelve receptors a reflex fall in blood pressure produced by injecting 8–25 μg veratridine (Bezold–Jarisch reflex) yielded results qualitatively similar to those following injection of sodium pentobarbitone.

4. In sixteen out of twenty-five chemoreceptors it was observed that ventilating the cat with 5·6–6·7 % CO_2 produced either no or little increase in activity; in nine receptors there was a clear increase in activity, which fell initially or was abolished after injecting a single dose of 20 mg sodium pentobarbitone.

5. In all seven chemoreceptors tested in seven deeply anaesthetized cats it was found that a larger dose (about 50–60 mg) of sodium pentobarbitone had no direct depressant effect on aortic chemoreceptor activity. It followed that the initial depressant effect of the much smaller doses of sodium pentobarbitone observed during hypoxic and hypercapnic stimulation (see above) must be due to reduction in the sympathetic outflow to the aortic bodies. This conclusion was supported by the results following injections of veratridine.

6. By comparing the present results with those reported previously it was concluded that the variations in the responses of aortic chemoreceptors during hypoxia and hypercapnia reported by different investigators could be partly due to variations in the level of sympathetic activity prevailing under different experimental conditions.

INTRODUCTION

It is known that the aortic chemoreceptors are stimulated by hypoxia and hypotension (Lee, Mayou & Torrance, 1964) and reduction in oxygen availability (Paintal, 1967). However, there is no agreement about the basic mechanism of stimulation (see Eyzaguirre, Fitzgerald, Lahiri & Zapata, 1983). Among other reasons, this could be due to some extent to considerable variation in the results obtained in different laboratories. Three examples relating to the activity recorded under hypercapnic, normoxic and hypoxic conditions will illustrate this variation.

First, with regard to hypercapnia, Paintal & Riley (1966) found that hypercapnia was not a stimulant for aortic chemoreceptors. This was confirmed by Sampson & Hainsworth (1972). It was reconfirmed by Fitzgerald (1976), Hanson, Rao & Torrance (1979) and Fitzgerald & Dehghani (1982) for steady-state conditions. However, Lahiri, Mulligan, Nishino & Mokashi (1979) concluded otherwise from their observations.

Secondly, under normoxic conditions, marked variation in the activity has been reported. For example, Paintal (1967) found that the activity in seventeen out of twenty-seven fibres was less than 0·1 impulses s^{-1}. Since this level of activity was much lower than that observed by Lee *et al.* (1964), he concluded that such differences could be due to differences in recording techniques employed, i.e. single-fibre preparations in his case (Paintal, 1967) and few-fibre ones in the case of Lee *et al.* (1964). However, this explanation cannot hold in the case of the data reported by Lahiri and his collaborators because they counted impulses in individual fibres (e.g. Lahiri *et al.* 1979; Lahiri, Nishino, Mokashi & Mulligan, 1980; Pokorski, Mokashi, Mulligan, Nishino & Lahiri, 1981). In fact in Table 1 of Pokorski *et al.* (1981) the activity in eleven fibres under normoxic conditions averaged 3·4 impulses s^{-1} and in Table 1 of Pokorski & Lahiri (1982) it averaged 2·8 impulses s^{-1} (eleven fibres). This contrasts markedly with the fact that under normoxic conditions the average in Paintal's twenty-seven fibres comes to only 0·4 impulses s^{-1} (see Table 1 of Paintal, 1967).

Finally, the data obtained in different laboratories during hypoxic stimulation provides equally strong contrasts. For example, Pokorski *et al.* (1981) found that the activity in eleven chemoreceptors averaged 12·6 impulses s^{-1} when the mean arterial P_{O_2} was 40 mmHg. On the other hand, from Table 2 of Paintal's (1971) data it can be seen that the activity in nine chemoreceptor fibres averaged 5·9 impulses s^{-1} at an arterial P_{O_2} of about 19 mmHg, i.e. the activity recorded was less than half that observed by Pokorski *et al.* (1981) even though the stimulus to the chemoreceptors was much greater, being 19 mmHg against the 40 mmHg arterial P_{O_2} of Pokorski *et al.* (1981).

From the above it is clear that the results obtained in different laboratories have differed widely, and the differences cannot be overlooked. Experiments were therefore conducted to find an explanation for the differing observations. The results obtained show that a major part of the differences may be due to the different levels of sympathetic activity prevailing in different sets of experiments conducted in the same or different laboratories. This is a plausible explanation, as the activity of aortic chemoreceptors is known to be influenced reflexly through sympathetic pathways (Lee *et al.* 1964; Anand, 1979).

METHODS

Experiments were carried out on cats anaesthetized with sodium pentobarbitone (Sagatal, May and Baker; 35 mg kg^{-1}) injected into the peritoneal cavity. Subsequently, small supplementary doses were injected intravenously whenever required, particularly for suppressing spontaneous respiration after the cat had been put on the respiratory pump. A rectal thermometer was inserted and the temperature of the cat maintained at about 37 °C.

Catheters were inserted into the right saphenous vein for injecting drugs, and into the right femoral artery for recording the aortic blood pressure; the latter was also used for withdrawing arterial blood samples in most experiments but in a few a separate catheter inserted in the left femoral artery was used. The blood pressure was recorded with a Statham type P23 Gb transducer. The upper part of the neck of the right side was prepared for dissecting and isolating fibres of chemoreceptors from the aortic nerve as described earlier (see Paintal, 1967; Anand, 1979). The right aortic nerve was cut centrally and the left vagus was cut in most experiments. A Tektronix type 122 or Iselworth type 102 preamplifier (bandpass > 5 kHz) was used for recording impulses from the chemoreceptor fibres with silver–silver chloride recording electrodes.

In one series of six cats a Fogarty embolectomy catheter number 5 was inserted into the right jugular vein so that its tip lay in the right atrium. This was used for distending the small balloon located at the tip of the catheter for obstructing venous return in order to produce a fall in blood pressure. In a second series of four cats the balloon-tipped catheter was made in our laboratory with latex rubber.

Hypoxic (4–8 % O_2 in N_2) and hypercapnic (5·6–6·7 % CO_2 and 21 % O_2 in N_2) gas mixtures were prepared and stored in gas cylinders under pressure. Their composition was analysed with a Scholander 0·5 ml gas analysis apparatus. These gas mixtures were delivered to the cat through a respiratory pump (Palmer, Ideal) via a gas bag. The resulting blood gas tensions in the samples of arterial blood and its pH were measured with a Radiometer PHM 27 equipment or an Instrument Laboratory Blood Gas Manager, Model 1312. Some samples had to be discarded as air bubbles got into the syringe inadvertently during collection.

Finally, after completing the preliminary procedures but before testing the first filament for chemoreceptor activity, artificial ventilation of the cat with a respiratory pump was begun. The rate of the pump was about 17 min^{-1} and suitable volumes (about 17 ml kg^{-1}) that suppressed spontaneous breaths in combination with adequate levels of general anaesthesia were used. This inevitably led to a fall in P_{a,CO_2} by a few millimetres of mercury but this small fall was of no consequence in the present experiments (see Results).

Transient inhibition of the vasomotor centre. Sudden reduction of the sympathetic outflow to systemic blood vessels was achieved by injecting a single dose of about 20 mg sodium pentobarbitone intravenously. This causes depression of the vasomotor centre, leading to reduction of the sympathetic outflow to the blood vessels (Goodman & Gilman, 1955*a*; Goth, 1979) with a consequent brief fall in blood pressure.

Identification of aortic chemoreceptor fibres. Filaments of aortic nerve fibres that fired randomly without a cardiac rhythm and which increased their activity considerably following ventilation with 4–8 % O_2, and in which the activity fell quickly on ventilating with air, were accepted as aortic chemoreceptor fibres. They were not selected on the basis of their response to hypotension, as one of the aims of the present investigation was to determine the responses of randomly selected aortic chemoreceptors to a fall in blood pressure. If the selected filament contained several chemoreceptor fibres then it was subdivided until a filament was obtained containing usually two active fibres with impulses clearly identifiable from one another on the basis of size and shape as seen on the monitoring oscilloscope (Tektronix type 422). Such filaments were used for the observations reported in the Results.

Recording procedure. The impulses, intratracheal pressure, aortic blood pressure and the time (using a Racal GRA 011 unit) were recorded on a 4DS or 7DS Racal tape-recorder with a bandpass of d.c. to 1200 Hz. Simultaneously, the blood pressure and the intratracheal pressure were monitored continuously on a direct ink-writing Beckman two-channel dynograph. After completing the experiment the physiological variables were played back from the tape-recorder into a Tektronix type 7704A oscilloscope and recorded on photographic paper (70 mm wide) with a continuously recording camera. The individual chemoreceptor impulses were simultaneously displayed on vertical sweeps (see Paintal, 1971) so as to establish the identity of each impulse in the case of filaments in which there were two or more active chemoreceptor fibres.

Analysis. The impulses of individual chemoreceptor fibres were averaged over 10 s and expressed as impulses s^{-1} as shown in Figs 1 and 5–9. The latencies for stimulation of the receptor (Figs 3 and 4) were obtained by plotting the activity every 2 s and from this estimating the start of increase in the activity. The error involved in these estimates could be about 0·5 s because of the irregular nature of the discharge. The paired *t* test was used for determining the significance of the difference between the activity of the chemoreceptors during normoxia and hypercapnia respectively.

RESULTS

The main results of the present investigation relate to the effects of reducing the sympathetic outflow from the vasomotor centre to the systemic blood vessels

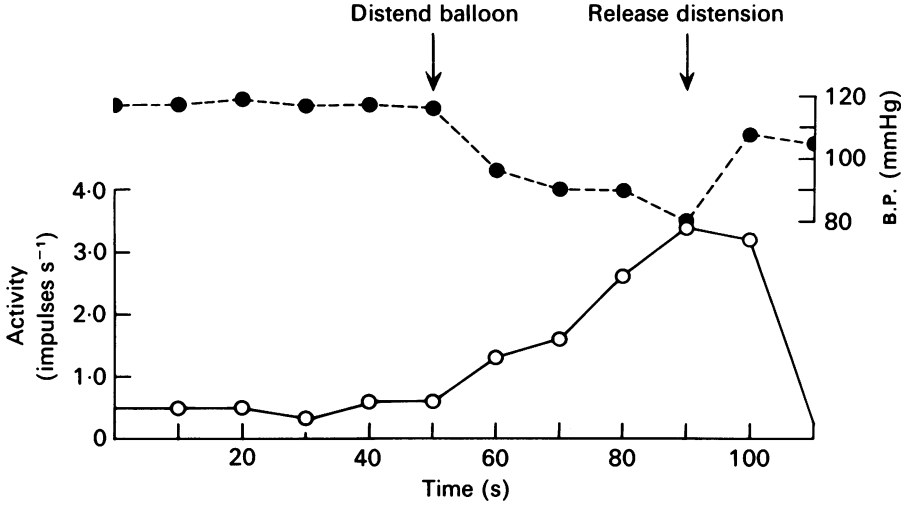

Fig. 1. Effect of mechanical hypotension, produced by distending a balloon in the right atrium at the arrow, on the activity of an aortic chemoreceptor; the activity was averaged over 10 s periods. The blood pressure (dashed line, ordinate on the right) fell by 36 mmHg and the latency for the increase in activity was about 2 s.

(including those of the aortic bodies) on the activity of chemoreceptors during hypoxia and hypercapnia. However, inhibition of the vasomotor centre inevitably involves a fall in blood pressure which is itself a stimulant for aortic chemoreceptors (Lee *et al.* 1964; Paintal, 1967; Lahiri *et al.* 1980). The effects of fall in blood pressure *without* inhibition of the sympathetic will therefore be described first in order that the effect of inhibition of the sympathetic (leading to a fall in blood pressure) on chemoreceptor activity can be meaningfully interpreted.

Effect of hypotension without sympathetic inhibition

A fall in blood pressure without inhibiting the sympathetic (but probably involving sympathetic stimulation) was produced mechanically by partially obstructing the venous inflow by distending a balloon in the right atrium (mechanical hypotension) while the cats were ventilated with air. The effect of this on the activity of sixteen chemoreceptors was recorded. The fall in the blood pressure was followed by an increase in the activity of all the sixteen chemoreceptors tested. An example

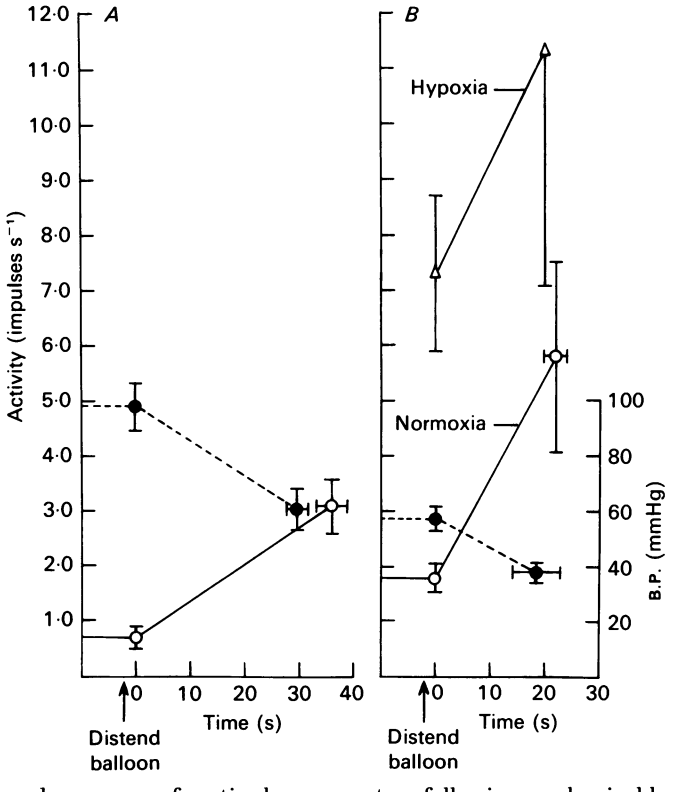

Fig. 2. Averaged responses of aortic chemoreceptors following mechanical hypotension in two series of experiments. In *A* the average initial blood pressure (●----●) was 98 mmHg (ordinate on the right of *B*). The continuous line (○——○) in *A* shows the increase in activity in sixteen chemoreceptors during normoxia following a mean fall in blood pressure of 37 mmHg: in *B* it shows a similar response in ten receptors from a second series of experiments in which the initial blood pressure of 57 mmHg was much lower than in *A* and so the initial activity was much higher in *B*. △——△: response of the same group of receptors (eleven) during hypoxia; their response during moderate hypercapnia is shown in Fig. 7*A*. The blood pressure values (●----●) can also apply to the hypoxia group. Bars, 1 S.E.M.

is shown in Fig. 1 and the averaged response at an initial average blood pressure of 98 mmHg is shown in Fig. 2*A*. Figure 2*B* shows the averaged response obtained from a second series of similar experiments involving ten receptors in which the initial blood pressure averaged 57 mmHg, i.e. it was much lower and therefore the control activity in the chemoreceptors before distending the balloon was higher.

This effect of hypotension, as well as the inverse relationship between arterial blood pressure and activity in the chemoreceptors, was expected from earlier observations (Lee *et al.* 1964; Paintal, 1967; Lahiri *et al.* 1980). However, so far the latency between the fall in blood pressure and the rise in activity has not been reported. This is shown in Fig. 3 which indicates that in 85% of the sample of twenty-six receptors (of both series) the activity started to increase within 6 s following the start of the fall in blood pressure. In no case was there a fall in activity even in fibres with spontaneous activity of about 1–4 impulses s^{-1}. The mean latency

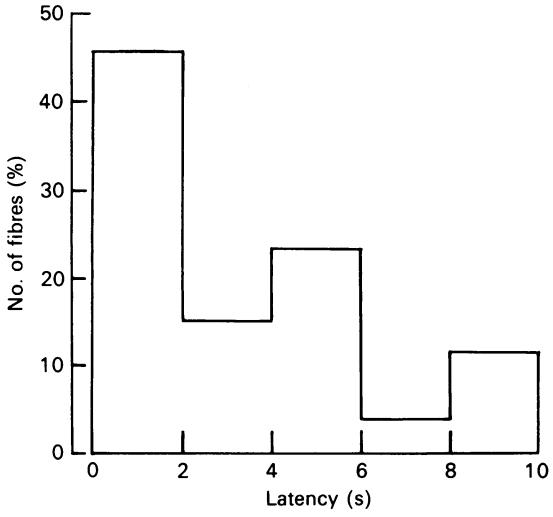

Fig. 3. Latencies (abscissa) for increase in activity in aortic chemoreceptors following mechanical hypotension during normoxia. The latencies represent the interval between the start of fall of blood pressure and the start of increase in activity above the control level. Ordinate, number of fibres (% of total = 26 from both series of experiments (see text)).

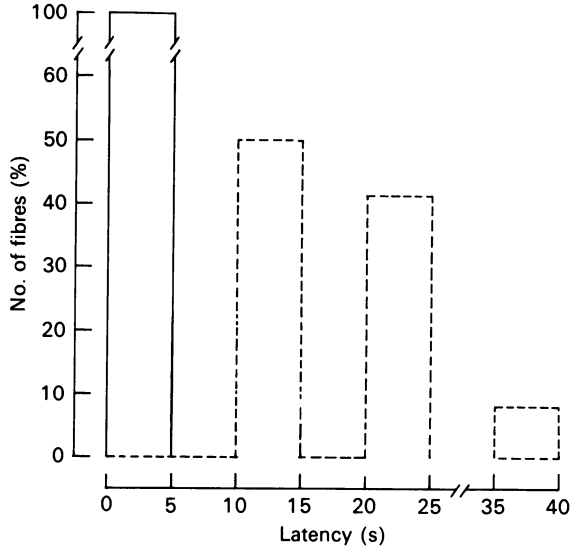

Fig. 4. Latencies (abscissa) for increase in activity in aortic chemoreceptors following mechanical hypotension (continuous lines) and pentobarbitone hypotension (dashed lines) during hypoxic stimulation. Ordinate, number of fibres (% of total) with different latencies; total number of fibres in mechanical hypotension group, 10; in pentabarbitone hypotension group, 12. The latter were obtained from a different set of experiments but the level of hypoxia was approximately the same in both groups.

for the increase in activity was 4·6 s (range, 2–10 s; S.E.M., 0·7) in the first series and 3·2 s (range, 1–10 s; S.E.M., 0·9 s) in the second series; the difference between the two means was not statistically significant. The mean fall in blood pressure was 37 mmHg (range, 25–56 mmHg; S.E.M., 2·3), the mean control level being 98 mmHg (range, 61–154 mmHg; S.E.M., 8·2) in the first series. In the second series the average fall in blood pressure was less, i.e. 19 mmHg (S.E.M., 4·2 mmHg). The rate of fall of blood pressure was also less, i.e. 1·1 mmHg s^{-1} compared to 1·4 mmHg s^{-1} in the first series. In spite of this the increase in the activity during hypotension was greater in Fig. 2*B*. This suggests that the initial blood pressure may play an important role.

The increase in activity was not due to fall in arterial P_{O_2}. In fact this was raised a little during the hypotensive period, as is to be expected (see Lahiri *et al.* 1980). Mean arterial P_{O_2} before and during hypotension were respectively 101 and 104 mmHg in the case of fourteen fibres of the first series.

Effect of mechanical hypotension during hypoxia and hypercapnia

In a separate group of four cats the effect of mechanical hypotension was examined on eleven receptors during normoxia, hypoxia and hypercapnia. The responses of all the receptors were qualitatively similar under all three conditions. Figure 2*B* shows the average response of the receptors during normoxia and hypoxia (the response during hypercapnia is shown in Fig. 7*A*). In Fig. 2*B* the blood pressure change, which shows the mean values during normoxia, also represents the blood pressure values during hypoxia as the values before and during hypotension were similar.

The mean latency for stimulation of the chemoreceptors measured from the start of the fall of blood pressure was 3·3 s (range, 1–6 s; S.E.M., 0·6 s) during normoxia, 2·6 s (range, 1–5 s; S.E.M., 0·4 s) during hypoxia and 2·7 s (range, 2–5 s; S.E.M., 0·4 s) during hypercapnia. The mean values were not significantly different from one another.

Effect of fall in blood pressure through inhibition of sympathetic outflow

As mentioned in the Methods a brief fall in blood pressure, by depressing the vasomotor centre, was produced by injecting a single dose of about 20 mg sodium pentobarbitone intravenously. The hypotension so produced will be referred to as pentobarbitone hypotension. Since preliminary experiments had shown that the initial fall in blood pressure was accompanied by a *reduction* in chemoreceptor activity instead of the increase described above, all the observations in this section were made after the level of the activity in one group of twelve chemoreceptors had been raised by ventilating the cat with 4–8% O_2 in N_2 (hypoxia subgroup) and in another group of nine exceptional receptors (see below) in which the activity had risen after ventilating the cat with about 5·6–6·7% CO_2 gas mixtures (hypercapnia subgroup).

Hypoxia subgroup

The effect of injecting about 20 mg sodium pentobarbitone was observed on twelve aortic chemoreceptors during hypoxia (4–8% O_2). In eight of them the activity fell within 10 s following the start of hypotension, i.e. at a time when one expected that the activity would rise (see Fig. 1). In three fibres there was no early change in

activity and in only one the activity rose from an initial value of 8·0 to 8·3 s^{-1} but the latency for even this small increase was 22 s.

After the initial fall, the activity returned to the control value, and thereafter the expected increase in activity above control value took place. The increase in activity after the initial fall can be assumed to be the direct effect of hypotension. The latencies for this, as shown in Fig. 4, were (because of the initial fall in activity) much

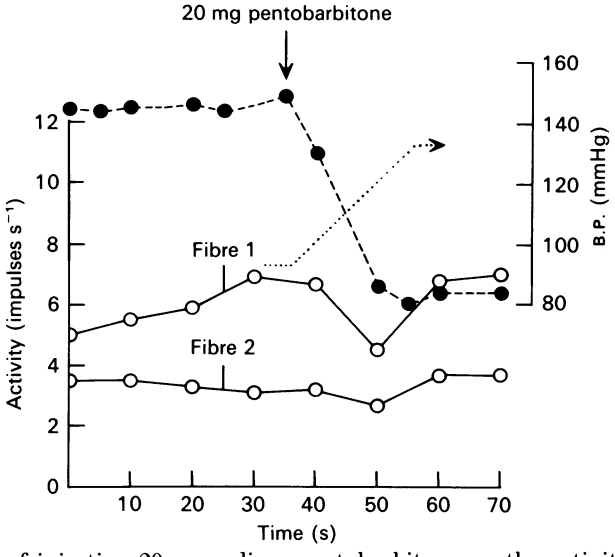

Fig. 5. Effect of injecting 20 mg sodium pentobarbitone on the activity of two aortic chemoreceptors stimulated by ventilating the cat with 4% O_2 (P_{a,O_2}, 31 mmHg; P_{a,CO_2}, 21 mmHg; pH, 7·420). After injection at the arrow the blood pressure fell but the activity of the two chemoreceptors, instead of rising, fell a little (fibre 2) or obviously (fibre 1). The P_{a,O_2} also fell during hypotension, i.e. 26 mmHg (P_{a,CO_2} was 20·2 mmHg and pH 7·477). The average increase in activity following mechanical hypotension during hypoxia is shown by the dotted line (see Fig. 2*B* for details). Blood pressure ●---●; activity, ○——○.

longer than that following mechanical hypotension during hypoxia. The mean latency for the increase (actually rise towards control value) in twelve fibres was 18·3 s (range, 11·0–37·0 s; S.E.M., 2·2), i.e. much greater than the mean value of 4·1 s following mechanical hypotension (i.e. without inhibition of the sympathetic) during normoxia (this difference was statistically significant, $P < 0·001$), or during hypoxia in which the mean was 2·6 s and the range was clearly much lower (see above).

The reduction in activity was not due to a possible rise in arterial P_{O_2} during the period of hypotension since this in fact fell by 3–5 mm (mean fall 3·4 mmHg) in the case of seven out of the twelve fibres for which P_{a,O_2} could be measured; in the case of one fibre the arterial P_{O_2} was unchanged during hypotension.

An example of the effect of inhibiting the sympathetic outflow is shown in Fig. 5. Here the administration of 4% O_2 raised the activity from 0·1 s^{-1} (not shown) to 6·9 and 3·2 impulses s^{-1} in two fibres respectively. At the arrow 20 mg sodium pentobarbitone was injected and within 8 s the discharge started to fall. Thereafter

the activity started to increase after a latency of about 15 s following the start of the fall in blood pressure. This, as well as the other results of this section, therefore suggests that part of the increase in activity produced by hypoxia must have been due to increased sympathetic activity (assumed to cause vasoconstriction of the vessels of the aortic bodies) which was reduced by sodium pentobarbitone, leading to an increase in O_2 availability with the consequent fall in activity (see Discussion).

The difference between these results and those following mechanical hypotension during hypoxia are striking, as shown in Figs 2*B* and 5.

Effect of hypercapnia

The effect of ventilating the cat with 5·6–6·7 % CO_2 hypercapnic gas mixtures on the activity of twenty-five aortic chemoreceptors was recorded and the impulses during the period of maximum activity 2–3·5 min after turning on the hypercapnic mixture counted over a 30 s stretch of the record. The mean activity of these receptors while ventilating with air was 0·8 impulses s^{-1} (range, 0·1–3·9 impulses s^{-1}; S.E.M., 0·2). During hypercapnia the mean rose to only 1·08 impulses s^{-1} (range, 0·2–4·3 impulses s^{-1}; S.E.M., 0·2). However, the difference between the means was significantly different using the paired *t* test for significance ($P < 0·001$).

The activity of these twenty-five receptors during hypercapnia has been plotted in Fig. 6 against their respective activity while ventilating the cat with air (abscissa). It can be seen that apart from one receptor in which the activity fell during hypercapnia and a few in which it did not change at all, the activity increased either by small amounts in most of them (fifteen receptors) or obviously in nine receptors. It should be noticed that the obvious increase in activity occurred in the nine receptors of Fig. 6 in which there was a noteworthy level of activity ($> 0·8$ impulses s^{-1}) during normoxia. The mean arterial P_{CO_2} while ventilating with air was 25·3 mmHg and it rose to a mean of 41·2 mmHg (range, 35–54 mmHg) during hypercapnia in the case of thirteen fibres in which the arterial P_{CO_2} could be estimated. This is the range of arterial P_{CO_2} in which the maximum rate of increase of activity was reported by Lahiri *et al.* (1979).

The increase in activity could not be attributed to a possible reduction in arterial P_{O_2} as this did not occur. In fact the average arterial P_{O_2} was a little higher during hypercapnia (mean increase in the case of thirteen fibres was 4·2 mmHg; range, -5 to $+13$ mmHg). This degree of change (assumed to apply also in the case of the remaining twelve receptors) could have had hardly any effect because, as shown already (Paintal & Riley, 1966), the chemoreceptor activity curve flattens out at normoxic levels.

From Fig. 6 it is clear that in the majority of chemoreceptors the excitatory effect of CO_2 was small. In those in which the increase was obvious (Fig. 6), it was felt that as in the case of hypoxic stimulation, the increased activity could be due to increased sympathetic activity. This conclusion was supported by the observations described below, following injection of about 20 mg sodium pentobarbitone.

Effect of pentobarbitone hypotension during hypercapnia

As in the hypoxia subgroup (see above), reducing the sympathetic outflow following injection of sodium pentobarbitone resulted in a fall in blood pressure

accompanied by an initial fall in the activity of nine chemoreceptors within 10 s of the start of fall in blood pressure. The reduction of activity was particularly striking in one cat in which activity was recorded from four chemoreceptor fibres simultaneously in one filament. Figure 7*B* shows the average activity in the four fibres. The activity was unusually high in them during normoxia and it increased

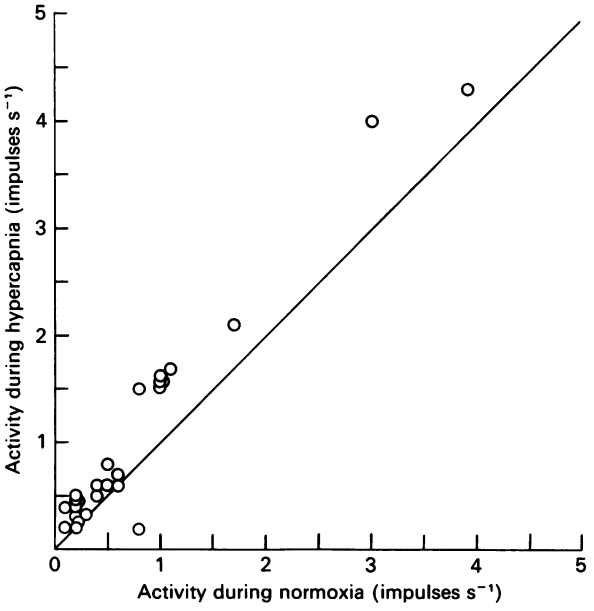

Fig. 6. Activity of twenty-five aortic chemoreceptors during hypercapnia (ordinate) plotted against their respective activities during normoxia (abscissa). The line passing through origin is the line of no change. Note that the activity during hypercapnia (produced by ventilating with 5·6–6·7 % CO_2) increased obviously in those receptors in which there was noteworthy initial activity during normoxia.

further after ventilating the cat with 6·7 % CO_2. After injecting sodium pentobarbitone the activity not only did not return to control level (as in the hypoxia subgroup) within the usual 40–60 s but it remained much below it thereafter. These observations therefore suggest that the increase in activity that occurs in some chemoreceptors following hypercapnic gas mixtures could be due to the prevailing increased level of sympathetic activity (increased further by hypercapnia) leading to vasoconstriction in the aortic bodies (see Discussion). Perhaps if a larger dose of sodium pentobarbitone had been injected in the case of the experiment shown in Fig. 7, the activity would have been reduced much further, or perhaps abolished. The difference between the effect of pentobarbitone hypotension (Fig. 7*B*) and mechanical hypotension during hypercapnia (Fig. 7*A*) is truly striking.

Effect of large dose of sodium pentobarbitone

At the end of seven experiments a relatively large dose (50–60 mg) of sodium pentobarbitone was injected in order to ascertain whether the anaesthetic had any direct depressant effect on the chemoreceptors. At the time of this injection the seven

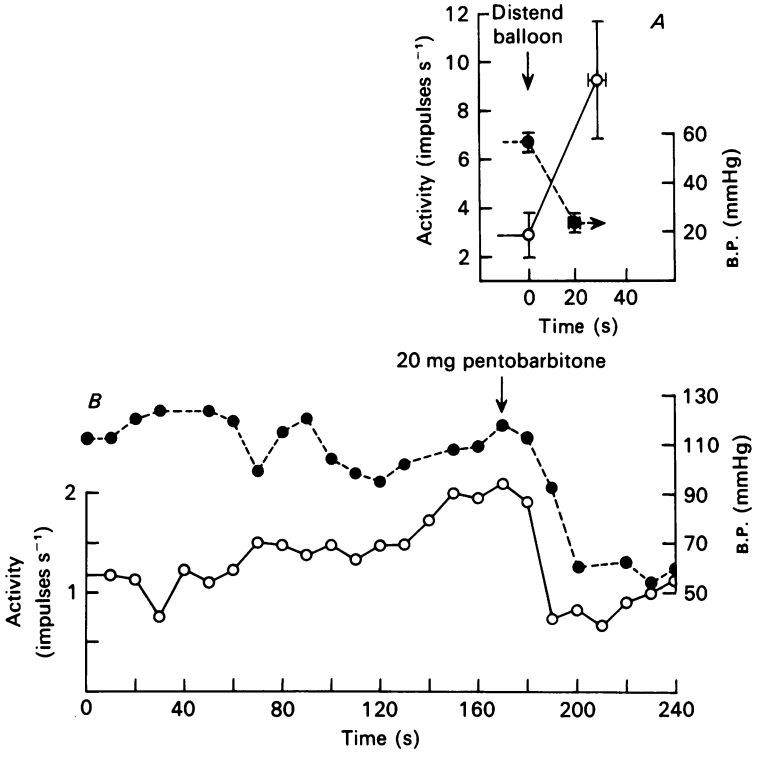

Fig. 7. Effect of pentobarbitone on chemoreceptor activity. *A* shows the average response (during hypercapnia) of eight of the eleven receptors following mechanical hypotension shown in Fig. 2*B* (hypoxia and normoxia). *B* shows the effect of injecting 20 mg sodium pentobarbitone on the average activity of four aortic chemoreceptors whose activity increased after ventilating the cat with 6·7 % CO_2 gas mixtures. These receptors had uncommonly high activity (at time zero) before the hypercapnia. *B* shows that after injecting sodium pentobarbitone the blood pressure fell by about 50 mmHg but instead of increasing as in *A*, the activity fell sharply and did not return to its initial level. The scale of the ordinate in *A* is four times that in *B* but the scale of the abscissa is the same in both *A* and *B*. Abscissa zero in *A* has been aligned with start of fall of blood pressure in *B*. Blood pressure, ●---●; activity, ○——○.

cats in which this was done had already received one or two injections of 20 mg sodium pentobarbitone earlier and were deeply anaesthetized. The blood pressure was low (in most cats < 60 mmHg) and apparently because of this there was a raised level of activity in the seven chemoreceptors even though the cats were ventilated with air. The injections of the large dose led to a drop in the blood pressure and this was followed by a marked increase in the activity of all the seven chemoreceptors. An example is shown in Fig. 8. The marked increase in activity shows specifically that sodium pentobarbitone does not have a direct local anaesthetic type of effect as local anaesthetics raise the threshold and reduce the frequency of discharge, i.e. desensitize sensory receptors (Paintal, 1964).

A noteworthy feature seen in Fig. 8 (also noted in some of the other receptors) is that there was no initial fall in activity of the kind shown in Figs 5 and 7, most probably because of the deeper level of anaesthesia. The absence of the initial fall in

activity shows that large doses of sodium pentobarbitone do not depress chemoreceptor activity, either due to a direct vasodilator effect on the vessels of the aortic body or due to a direct effect on the chemoreceptor cells themselves leading to reduction in impulse generation. It follows that the initial depression of activity by the much smaller doses described above (Figs 5 and 7) could not be due to any direct depressant action through any of the mechanisms considered above. However, in

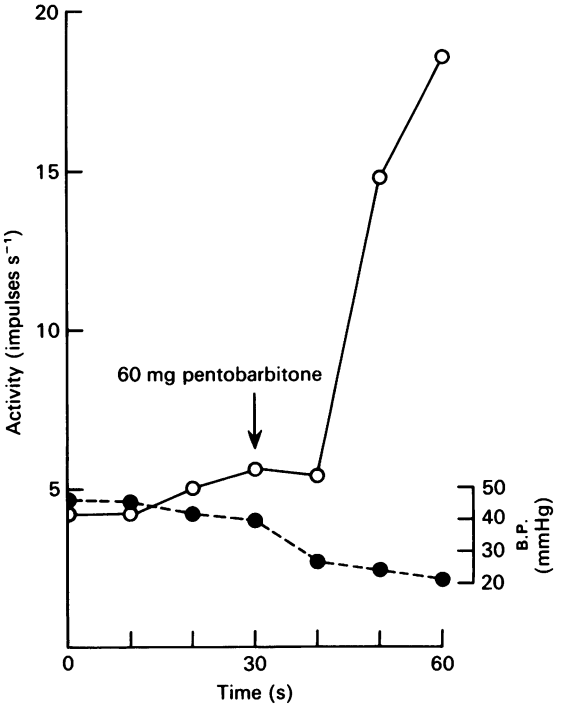

Fig. 8. Effect of injecting a relatively large dose (60 mg) of sodium pentobarbitone at the arrow on the activity of an aortic chemoreceptor. Note, to begin with the cat had been deeply anaesthetized. This lowered the blood pressure, thereby increasing the activity which varied around 5 impulses s^{-1}. The injection of the 60 mg dose of sodium pentobarbitone was followed by a further marked increase in the activity of the chemoreceptor owing to the fall in blood pressure to a very low level; note absence of initial fall in activity seen in Figs 5 and 7 with much smaller dose. Blood pressure, dashed line (ordinate on the right). The results show that sodium pentobarbitone has no local anaesthetic or vasodilator effects on aortic chemoreceptors.

order to rule out such factors experiments were also done using another method of reducing the sympathetic outflow, i.e. through the Bezold–Jarisch reflex which produces reflex hypotension through reflex reduction in the sympathetic outflow without a fall in cardiac output (Goodman & Gilman, 1955*b*).

Effect of the Bezold–Jarisch reflex

The effect of the Bezold–Jarisch reflex elicited by injecting veratridine (Sigma; 8–25 μg I.V.) was examined on twelve chemoreceptors: six during hypoxia (ventilation with 4% O_2) and six during moderate hypercapnia (ventilation with 5·6–6·7% CO_2).

All the injections of veratridine were followed by a fall in blood pressure ranging from 27 to 64 mmHg and this fall was accompanied by an initial fall in the activity of eight of the twelve chemoreceptors, as seen in the case of pentobarbitone hypotension (Figs 5 and 7). An example following veratridine injection is shown in Fig. 9; note the delayed increase in activity. In seven of these receptors it was confirmed that the fall in activity was not due to a rise in arterial P_{O_2}; in the case of

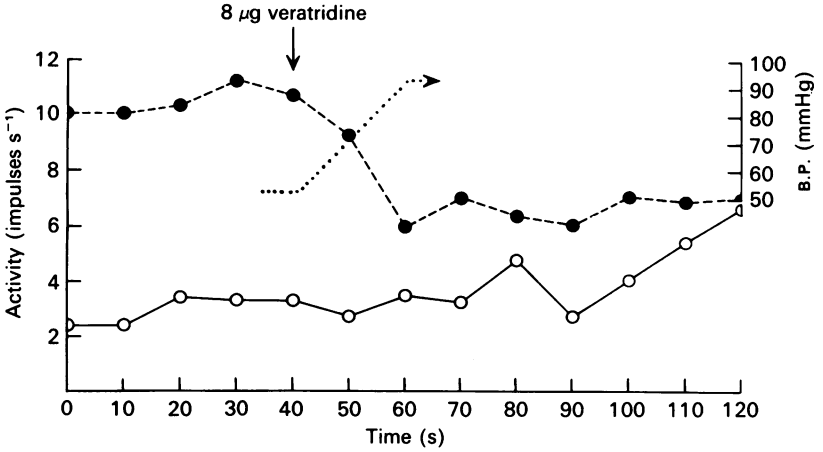

Fig. 9. Effect of injecting 8 μg veratridine (Bezold–Jarisch reflex) on the activity of an aortic chemoreceptor after it was stimulated by ventilating the cat with 4% O_2 (P_{a,O_2}, 26 mmHg; P_{a,CO_2}, 18·5 mmHg; pH, 7·335). After injection at the arrow the blood pressure fell and the activity, instead of increasing as shown by the dotted line, fell by 1 impulse s^{-1}. During hypotension the P_{O_2} remained practically unchanged, i.e. 25 mmHg (P_{a,CO_2}, 18 mmHg; pH, 7·330). The average increase in activity following mechanical hypotension during hypoxia is shown by the dotted line (see Fig. 2*B* for details). Symbols as in Fig. 7.

one receptor the blood gas tension could not be measured. In the remaining four receptors there was no initial fall in activity before the usual increase following hypotension. The latency for this increase in activity ranged from 10 to 50 s (mean 18·3 s; S.E.M., 6·4) in the hypoxia group, and from 10 to 45 s (mean, 27 s; S.E.M., 5·4) in the hypercapnia group. These values, which are closer to those obtained during pentobarbitone hypotension (mean, 18·3 s), are much longer than those following mechanical hypotension during hypoxia (mean, 3·6 s), hypercapnia (mean, 2·7 s) and normoxia (mean, 3·3 s) in one group of ten receptors and 4·6 s in a second group of sixteen receptors during normoxia (see also Fig. 3).

Since veratrum alkaloids stimulate carotid chemoreceptors directly (see Heymans & Neil, 1958) the initial reduction of chemoreceptor activity through a possible depressant effect on aortic chemoreceptors by veratridine is unlikely. The initial depression in the eight receptors described above can therefore be attributed, as in the case of pentobarbitone hypotension, to a reduction of the sympathatic outflow to the vessels of the aortic body through the Bezold–Jarisch reflex.

DISCUSSION

The conclusions (see below) that follow from the results are based on the following three assumptions: (1) that activity of aortic chemoreceptors is influenced by

activity in sympathetic efferent fibres that regulate the diameter of the blood vessels of the aortic bodies, (2) that sodium pentobarbitone (50–60 mg) has no *direct* depressant action on aortic chemoreceptor activity, and consequently (3) that intravenous injections of small doses (20 mg) of sodium pentobarbitone must produce their depressant effect on aortic chemoreceptor activity solely through reduction of sympathetic activity to the vessels of the aortic bodies.

The first assumption is valid in view of the earlier observations of Mills (1968), Lee *et al.* (1964) and Anand (1979). The validity of the second assumption has been unequivocally demonstrated in the present investigation (Fig. 8). Thus the third assumption also becomes valid as there is no other way in which sodium pentobarbitone could depress chemoreceptor activity. Even the remote possibility of influence through the efferents of Neil & O'Regan (1971) is ruled out since the right aortic nerve was cut in all experiments and the left also cut along with the vagus in most experiments. In view of the above it follows that immediate reduction in the activity of aortic chemoreceptors following intravenous injection of a small dose of sodium pentobarbitone (Figs 5 and 7) must be due to the reduction of existing activity in sympathetic efferent fibres to the aortic bodies, leading to an increase in O_2 availability to the chemoreceptors in them. This conclusion is strongly supported by the similar effects produced by the Bezold–Jarisch reflex (Fig. 9). These results imply that there must be a considerable contribution through the sympathetic outflow to the stimulation of the chemoreceptors while ventilating the cat with hypoxic and hypercapnic gas mixtures. Specifically, this means that during hypoxia there must be an increase in the sympathetically produced glomeral vasoconstriction. This was not unexpected in view of the known excitatory effects of hypoxia on the sympatho-adrenal system (Euler, 1956). Thus the inevitable conclusion follows that at present one cannot obtain a valid plot of the relation between arterial P_{O_2} and chemoreceptor activity owing to the unknown amount of sympathetic activity which is bound to be present even under normoxic conditions and which would increase by unknown amounts during various degrees of hypoxia. This would inevitably involve a shift of the P_{O_2}–activity curve to the right. Hence at present one can have only qualitative information regarding the relationship of any chemoreceptor stimulus (e.g. arterial P_{O_2}, blood pressure or O_2 content) to the activity of the aortic chemoreceptors. One could conceivably eliminate sympathetic activity either by pharmacological means or by cutting the sympathetic fibres to the aortic bodies (see Mills, 1968). The first method cannot produce total block without serious side effects, and cutting the sympathetic nerves would involve opening the chest and exposing the aortic bodies to the atmosphere which, as already demonstrated, has its own disadvantages (Anand & Paintal, 1980). With the aortic bodies exposed to air it would not be possible to obtain a quantitatively valid relation of arterial P_{O_2} (or blood pressure) to the activity of the receptor as the local tissue P_{O_2} would be influenced by the O_2 entering the aortic body from outside (Paintal, 1968).

The present observations on the effects of hypercapnia on aortic chemoreceptor activity help to resolve the differences in the observations (and conclusions) from different laboratories (see Introduction), as it now seems that the greater excitation, dynamic or under steady-state conditions, observed by Lahiri *et al.* (1980) may be due to the probably greater sympathetic activity in their experiments since their cats

appear to have been more lightly anaesthetized (chloralose, 60 mg kg^{-1}) as compared to the level of anaesthesia obtaining in experiments done earlier by Paintal & Riley (1966) in which chloralose at 75 mg/kg^{-1} was used and no neuromuscular block was necessary to supress spontaneous respiratory movements. On the other hand, Lahiri and his co-workers (Lahiri *et al.* 1979; Pokorski *et al.* 1981; Pokorski & Lahiri, 1982) have had to consistently use neuromuscular block. Under such circumstances the sympathetic outflow could be considerable, thus accounting for the more frequent, though small (see Eyzaguirre *et al.* 1983) increase in activity of the aortic chemoreceptors during hypercapnia observed by Lahiri *et al.* (1980). This conclusion is supported by the results shown in Fig. 6 which show that the obvious increase in activity occurred in those receptors in which there was noteworthy activity ($< 0{\cdot}8$ impulses s^{-1}) to begin with during normoxia, perhaps due to greater sympathetic tone in the vessels of the aortic bodies.

It should be noted that some of the differing conclusions relating to the effect of hypercapnia can be attributed to the greater importance given to small increases in activity by Lahiri *et al.* (1980) than by others. This fact is now recognized by Lahiri and his co-workers, as the following statement by them (Mulligan, Lahiri, Mokshi, Matsumoto & Mcgregor, 1986) would indicate: 'In any event the effect of P_{a,CO_2} change on aortic chemoreceptor activity is relatively small (see Eyzaguirre *et al.* 1983)'. However, activity can increase during hypercapnia in some receptors, but that increase is most probably due to sympathetic vasoconstriction of the vessels of the aortic bodies. In any case, the mean increase in activity from 0·8 impulses s^{-1} (during normoxia) to 1·1 impulses s^{-1} during hypercapnia is insignificant when compared to the increase in activity observed during hypoxia. For example, in one set of observations on twenty-seven chemoreceptors (Paintal, 1967) the activity increased from 0·4 impulses s^{-1} during normoxia to about 8·1 impulses s^{-1} following ventilation of the cat with 4% oxygen.

As in the case of hypercapnia (see above), it would be reasonable to also attribute the large differences in the level of activity in aortic chemoreptors recorded during normoxia and hypoxia by different investigators (see Introduction) to the differing levels of prevailing sympathetic activity. However, this cannot be the entire explanation as far as hypoxia is concerned because the levels of activity during hypoxia reported from different laboratories differ widely. For example, Pokorski *et al.* (1981) obtained a mean activity of 13 impulses s^{-1} at an arterial P_{O_2} of 40 mmHg. This level of activity is very high when compared with the activity recorded at 0 mmHg tissue P_{O_2} (i.e. much higher stimulus intensity) after circulatory arrest, which amounted to only 9 impulses s^{-1} (see Table 2 of Paintal, 1971). Perhaps differences in the techniques used for identifying single fibres might account for the differences. Indeed, as also noted in the present investigation, it is not uncommon to have two or more fibres with the same spike height existing in the same filament, a fact that can be established by displaying the shapes of individual impulses on photographic records (Paintal, 1971). This would be missed by the use of window discriminators which are commonly used (e.g. Pokorski *et al.* 1981), resulting in apparently higher recorded frequencies of discharge. It is noteworthy that using the improved system for displaying the shapes of individual impulses (Paintal, 1971), the mean activity while ventilating the cat with 4% O_2 was 5·9 impulses s^{-1} (see Table 2 of Paintal,

1971) whereas the mean activity was 8·1 impulses s^{-1} (i.e. about 37% more) in the earlier study (see Table 1 of Paintal, 1967) in which a less satisfactory system was used.

The results have shown that the effects of hypotension on aortic chemoreceptors depend on how the hypotension is produced. If it is produced mechanically by distending a balloon in the right atrium, then the result is an early increase in chemoreceptor activity (Fig. 1). Such a procedure, which mimics haemorrhage, would inevitably involve stimulation of the sympatho-adrenal system, including the fibres to the aortic bodies. On the other hand, if the hypotension is produced by inhibition of the vasomotor centre, e.g. by sodium pentobarbitone or veratridine, then the immediate effect is reduction of aortic chemoreceptor activity (Figs 5, 7 and 9), which can be attributed to some reduction of the existing activity in the sympathetic fibres to the aortic bodies. Since the effect of hypotension on aortic chemoreceptor activity depends on whether the hypotension is accompanied by reduction or increase in sympathetic activity, it follows that the same would apply in other situations. For example, releasing the clamps on previously clamped carotid arteries would reflexly lower systemic blood pressure but this hypotension would initially be expected to be accompanied by reduction in aortic chemoreceptor activity, resulting in a delayed increase in chemoreceptor activity.

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