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RESTORATION OF HYPOXIC RESPIRATORY RESPONSES IN THE AWAKE RAT AFTER CAROTID BODY DENERVATION BY SINUS NERVE SECTION

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SUMMARY

1. The restoration of ventilatory responses to hypoxia after carotid body denervation was studied in twenty-eight awake rats.

2. The respiratory depression seen in moderate hypoxia (partial pressure of inspired O_2, P_{I,O_2}, 80–100 mmHg) 3 days after bilateral carotid sinus nerve section disappeared by day 10. By day 17 respiratory stimulation occurred at all levels of P_{I,O_2} below 125 mmHg. The largest restored response, in severe hypoxia (P_{I,O_2} 50–60 mmHg), was approximately 55% of the pre-denervation response. The response showed little further change from day 17 to day 192.

3. A comparison of the effect of bilateral section of the glossopharyngeal nerve and of the abdominal vagus 1 and 28 days after carotid sinus nerve section demonstrated that the restoration of hypoxic response resulted in part from an enhanced effect of the inputs from the secondary glomus tissue served by these nerves.

4. A comparison of the effect of bilateral section of glossopharyngeal, abdominal vagal and aortic depressor nerves 1 and 28 days after carotid sinus nerve section demonstrated an increase of a residual hypoxic response which must result either from inputs from unidentified peripheral chemoreceptors or from central mechanisms.

5. Bilateral sectioning of the aortic depressor nerves produced no additional effect on restored responses to sectioning glossopharyngeal and abdominal vagal nerves, providing further evidence against significant aortic body function in the rat.

6. The studies support the hypothesis that central neural reorganization provides compensation for loss of carotid body function by enhancement of effects of normally subsidiary inputs.

INTRODUCTION

The loss of ventilatory sensitivity to hypoxia that follows denervation of the carotid bodies is subsequently partially restored in ponies (Bisgard, Forster & Klein, 1980) cats (Smith & Mills, 1980) and rats (Breslav & Konza, 1975; Sinclair, Laughton, German & Robson, 1980). The time course and the final extent of restoration have not been demonstrated. Progressive increases in the ventilatory

responses to carotid sinus nerve (c.s.n.) and aortic nerve stimulation after carotid body denervation in cats were regarded by Majumdar, Smith & Mills (1982) as evidence that the basis of restoration of response was a central synaptic reorganization of chemoreflex pathways. They proposed that regenerative proliferation of central synapses following atrophy enhanced the effects of residual chemoreceptor input. Evidence for such enhancement was provided in studies on ponies (Forster, Pan, Bisgard, Kaminski, Dorsey & Busch, 1983) in which restored responses were eliminated by sectioning the aortic nerves or by destroying the aortic bodies.

The rat is a suitable animal in which to study these phenomena. The major hypoxic input from carotid bodies can be eliminated by c.s.n. section, leaving further small hypoxic influences conveyed in the glossopharyngeal and abdominal vagal nerves (Martin-Body, Robson & Sinclair, 1985). Preliminary studies showed evidence for partial restoration of hypoxic responses, in that the depression of the ventilatory response to hypoxia that occurred immediately after bilateral c.s.n. section was converted at 6 weeks to a modest hyperventilation (Sinclair *et al.* 1980). The studies to be described were planned to characterize the restoration of hypoxic stimulation of breathing in the awake rat following bilateral c.s.n. section. The studies aimed to show the time course of restoration, its final extent, and the extent of its dependence on the secondary hypoxic inputs conveyed in the glossopharyngeal and abdominal vagal nerves. The absence of an influence of aortic bodies in the rat (Sapru & Kreiger, 1977; Barker, Easton & Howe, 1980) was tested. Hypoxic responses in the absence of all known peripheral chemoreceptor inputs also allowed evaluation of a possible central stimulation of breathing during hypoxia in the awake animal.

METHODS

The experiments were performed on female Charles-Wistar rats aged 92–110 days and weighing 214–310 g at the time of the first studies. Anaesthetic and surgical procedures have been described previously (Martin-Body *et al.* 1985). Denervation consisted of bilateral sectioning of the c.s.n. or the glossopharyngeal nerve (n.IX) in the neck, of the abdominal vagus nerve (n.Xa) just below the diaphragm and of the aortic depressor nerve (a.d.n.) close to its junction with the superior laryngeal nerve. Modifications of the plethysmographic technique (Bartlett & Tenney, 1970) by which tidal volume (V_T), respiratory frequency (f), and minute volume ($\dot{V}_E$) were measured in the awake animal have been described previously (Martin-Body *et al.* 1985; Martin-Body & Sinclair, 1985). The rat was held lightly restrained in the plethysmograph chamber in an atmosphere of air for a preliminary period of 30 min, after which duplicate respiratory recordings were made. Thereafter 10 min were allowed between the introduction of each hypoxic gas mixture and respiratory measurements; the box was then flushed again with the test gas for 2 min and the measurement repeated. Measurements were made after exposure to nine hypoxic mixtures. In early experiments, different mixtures were applied in random sequence to four rats on eight separate occasions. Results from these thirty-two sets of experiments, each giving nine duplicated measurements, showed no differences from results in age-matched controls to which mixtures were applied in order of diminishing O_2 concentration. The latter order was used in later experiments; all comparisons involved groups subjected to the same protocol.

Analyses of V_T, f, and $\dot{V}_E$ were made from twenty to thirty consecutive breaths in the final 30 s of each test period. Sighs and movement artifacts were excluded. For statistical analysis, data from all animals within a group were pooled and placed according to the partial pressure of the inspired O_2 (P_{I,O_2}) in bins for 45–60, 60–70, 70–80, 80–90, 90–100, 100–110, 110–130, and greater than 145 mmHg. An analysis of variance was performed for each bin using all experimental groups. If the F statistic for the bin was significant, a protected t test was used on pairs of groups. Thus, all statistics quoted represent the results of a protected t test. Differences were considered significant if P was less than 0·05.

In the first series of experiments, rats were studied at various intervals up to 192 days after bilateral c.s.n. section. In further experiments, bilateral c.s.n. section was performed, the efficacy of denervation was established by loss of f response to hypoxia 1 day later, and bilateral section of n.IX, n.Xa and a.d.n. in various combinations was undertaken 1–30 days later.

RESULTS

Restoration of hypoxic sensitivity 3–192 days after bilateral c.s.n. section

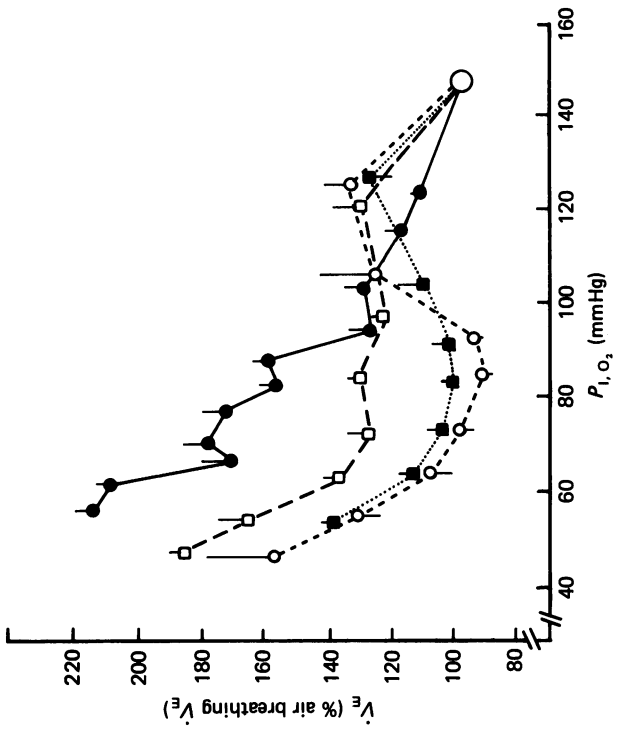
The ventilatory responses to multiple levels of hypoxia were measured at intervals of 3, 10, 17, 24, 31, 38 and 192 days after bilateral c.s.n. section in four rats and were compared with those from twenty-three studies of intact controls. These rats showed the expected age-related increase in V_T over the 6 month period of study (Martin-Body & Sinclair, 1985); the mean V_T when breathing air increased from $1{\cdot}67 \pm 0{\cdot}13$ ml (S.D.) to $3{\cdot}39 \pm 0{\cdot}25$ ml. Frequency when breathing air showed no significant change (85 ± 3 at day 3, 83 ± 3 at day 192). In these serial studies, to compensate for age-related changes in V_T and therefore in $\dot{V}_E$, respiratory variables during hypoxia were calculated in each study as percentages of the values obtained in air during the same study.

Details of the results are shown in Fig. 1. In the normal awake control rats, $\dot{V}_E$ increased progressively as P_{I,O_2} was reduced from air level to the lowest levels testable, P_{I,O_2} 45–55 mmHg. The increased ventilation resulted from progressive increases of frequency at P_{I,O_2} levels of 120–40 mmHg and increases of V_T at P_{I,O_2} levels of 90–40 mmHg.

Three days after bilateral c.s.n. section, respiration showed a triphasic response to hypoxia: a phase of stimulation in mild hypoxia, so that at P_{I,O_2} 120 mmHg $\dot{V}_E$ was higher than in intact controls subject to comparable hypoxia; a phase of depression of P_{I,O_2} was reduced to 75 mmHg, where $\dot{V}_E$ was less than in air; and a second phase of stimulation as P_{I,O_2} was reduced to the final mean level of 48 mmHg. The maximum $\dot{V}_E$ at P_{I,O_2} 45–50 mmHg was 156% the level in air whereas in the intact controls the maximum $\dot{V}_E$ at P_{I,O_2} 50–60 mmHg was 222% the level in air. The responses in $\dot{V}_E$ resulted from consistent variations in responses of both frequency and tidal volume. The increase of $\dot{V}_E$ in mild hypoxia resulted from an increase of V_T to 134% the air level at P_{I,O_2} 110–120 mmHg; at this stage the normal frequency response was absent. In moderate hypoxia, the V_T response diminished, and was 102% air level at P_{I,O_2} 90–100 mmHg, but f was below that in air and well below levels seen in controls. In more severe hypoxia, the increase of $\dot{V}_E$ resulted mostly from increase of V_T (to 139% air level, compared with 143% in controls) but frequency also increased, to 111% the air level (compared with 166% in controls).

At day 10, the ventilatory response remained triphasic, with the same increases as at day 3 in V_T and $\dot{V}_E$ in mild hypoxia and in V_T, f and $\dot{V}_E$ in severe hypoxia, but mean ventilation at P_{I,O_2} 80–100 mmHg was no longer depressed below normoxic levels because the depression of frequency at this level of hypoxia was now inconsistent.

At days 17, 24, 31 and 38, $\dot{V}_E$ increased at all levels of hypoxia tested. V_T responses were generally unchanged. The restoration of response resulted from the presence of a frequency response at all hypoxic levels. At P_{I,O_2} 48–49 mmHg the frequency response increased by day 38 to 144% the air value, compared with 166% in intact controls at mean P_{I,O_2} of 53 mmHg.



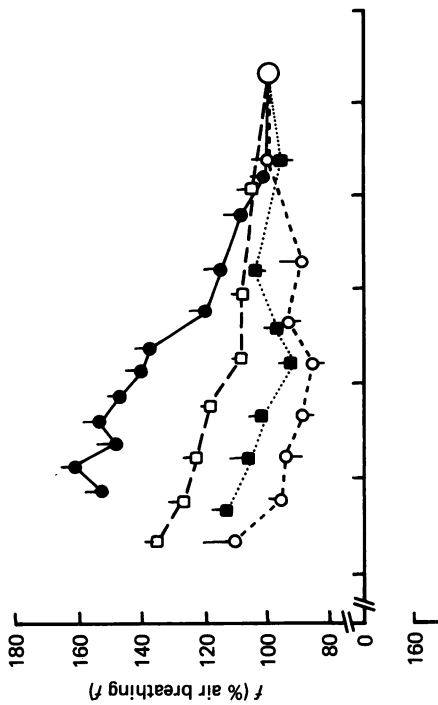


Fig. 1. Respiratory frequency (f), tidal volume (V_T) and minute volume ($\dot{V}_E$) at different levels of inspired oxygen (P_{I,O_2}), values expressed percentage values breathing air, in four rats before bilateral carotid sinus nerve section (●) and 3 days (○), 10 days (■) and 17–192 days (□) afterwards. Vertical bars show S.E. of mean.

At day 192 the responses were generally the same as at days 17–38. The consistency of results was such that the responses for all observations from days 17 to 192 fell within the narrow distribution shown in Fig. 1.

The effect of sectioning n.IX on the restored response to hypoxia

Hypoxic responses were tested in four rats 28 days after carotid body chemodenervation by bilateral c.s.n. section. Bilateral n.IX section was then performed and the animals restudied, awake, 1 day later. n.IX section converted the restored

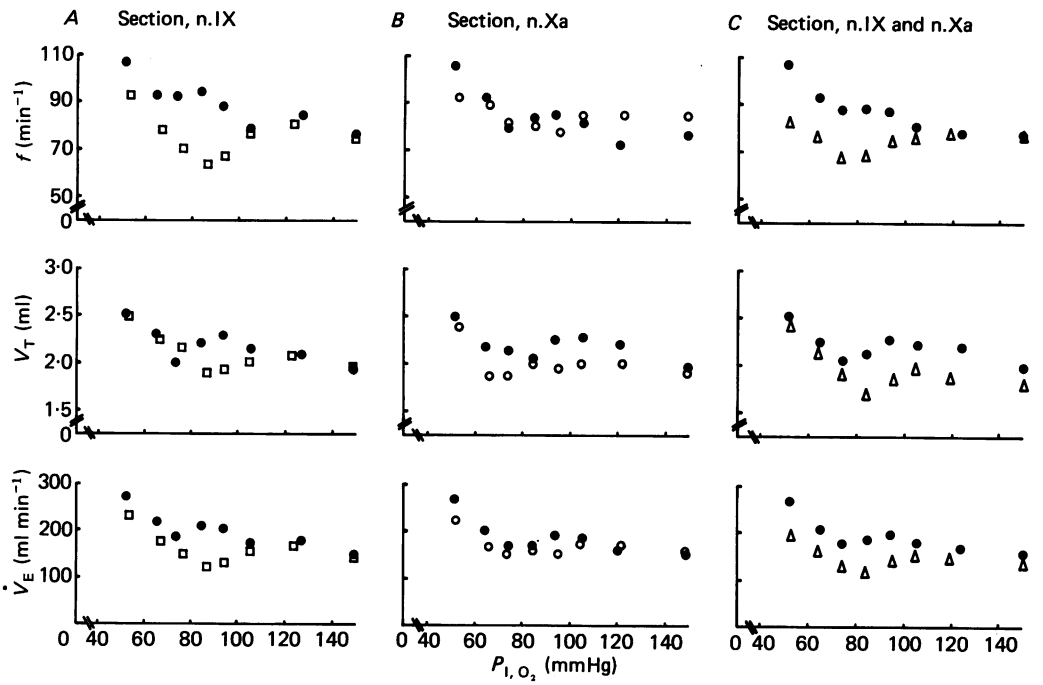


Fig. 2. The effects of bilateral section of (*A*) the glossopharyngeal nerves, n.IX (*B*) the abdominal vagi, n.Xa and (*C*) both, on the restored hypoxic response. Values of f, V_T and $\dot{V}_E$ at different levels of P_{I,O_2} 28 days after bilateral c.s.n. section (●), 29 days after c.s.n. section and 1 day after section of n.IX in four rats (□), of n.Xa in four rats (○) and 1–2 days after section of both n.IX and n.Xa in eight rats (△). For statistics, see text and table.

ventilatory response at P_{I,O_2} 75–105 mmHg to a depression, mostly from an effect on the restored frequency response. In more severe hypoxia, at P_{I,O_2} 52 mmHg, the restored response was diminished, f increased to 93 breaths min^{-1} compared with 107 breaths min^{-1} before n.IX section ($P < 0{\cdot}05$). n.IX section also removed the V_T response which was present 28 days after c.s.n. section at P_{I,O_2} 80–110 mmHg so that V_T remained close to the air value (1·97 ml) until P_{I,O_2} was below 76 mmHg. At P_{I,O_2} 53 mmHg V_T was 2·49 ml compared with 2·52 ml before n.IX section. Detailed results are shown in Fig. 2 and detailed statistics are given in Table 1.

TABLE 1. Effect of nerve section procedures on frequency, f (min^{-1}), tidal volume, V_T (ml) and minute ventilation, $\dot{V}_E$ (ml min^{-1}) (mean ± S.E. of mean) in rats subjected to c.s.n. section 30 days previously

P_{I,O_2} (mmHg)	n.IX section		n.Xa section		n.IX + n.Xa section	
	Before	After	Before	After	Before	After
	f	f	f	f	f	f
150	77 ± 2	75 ± 3	79 ± 2	85 ± 5	77 ± 2	76 ± 2
110–130	85 ± 5	81 ± 4	72 ± 2	85 ± 3**	77 ± 3	77 ± 2
100–110	79 ± 3	77 ± 3	82 ± 3	85 ± 4	80 ± 2	75 ± 3
90–100	88 ± 4	67 ± 3**	85 ± 2	77 ± 3*	87 ± 2	73 ± 3***
80–90	94 ± 8	64 ± 3**	83 ± 3	80 ± 4	88 ± 4	67 ± 2***
70–80	92 ± 8	71 ± 5*	79 ± 3	80 ± 5	87 ± 5	66 ± 2**
60–70	93 ± 5	78 ± 6	92 ± 6	89 ± 5	93 ± 4	75 ± 4**
45–60	107 ± 4	93 ± 4*	106 ± 4	92 ± 4*	106 ± 3	81 ± 2***
	V_T	V_T	V_T	V_T	V_T	V_T
150	1·93 ± 0·09	1·97 ± 0·09	1·98 ± 0·04	1·91 ± 0·05	1·97 ± 0·6	1·77 ± 0·06**
110–130	2·10 ± 0·08	2·08 ± 0·03	2·21 ± 0·05	2·01 ± 0·06*	2·17 ± 0·04	1·86 ± 0·05**
100–110	2·16 ± 0·07	2·01 ± 0·07	2·28 ± 0·06	2·10 ± 0·05**	2·21 ± 0·05	1·96 ± 0·03**
90–100	2·28 ± 0·07	1·93 ± 0·03***	2·26 ± 0·07	1·96 ± 0·04**	2·27 ± 0·05	1·85 ± 0·04**
80–90	2·20 ± 0·11	1·90 ± 0·03*	2·06 ± 0·05	1·99 ± 0·04	2·12 ± 0·05	1·68 ± 0·03***
70–80	1·99 ± 0·10	2·16 ± 0·07	2·14 ± 0·11	1·88 ± 0·05*	2·05 ± 0·07	1·90 ± 0·04
60–70	2·31 ± 0·04	2·24 ± 0·05	2·19 ± 0·07	1·89 ± 0·06**	2·25 ± 0·04	2·12 ± 0·06
45–60	2·52 ± 0·06	2·49 ± 0·08	2·51 ± 0·06	2·39 ± 0·06	2·52 ± 0·04	2·40 ± 0·08
	$\dot{V}_E$	$\dot{V}_E$	$\dot{V}_E$	$\dot{V}_E$	$\dot{V}_E$	$\dot{V}_E$
150	148 ± 7	147 ± 6	153 ± 6	157 ± 9	152 ± 8	131 ± 4***
110–130	176 ± 1	166 ± 8	160 ± 6	170 ± 6	166 ± 6	142 ± 5**
100–110	170 ± 9	154 ± 5	186 ± 9	170 ± 12	177 ± 6	149 ± 7**
90–100	200 ± 12	130 ± 6***	192 ± 9	150 ± 4***	196 ± 7	137 ± 6***
80–90	206 ± 17	121 ± 7***	170 ± 6	159 ± 8	185 ± 9	112 ± 5***
70–80	183 ± 20	149 ± 9	169 ± 14	149 ± 8	178 ± 13	125 ± 4***
60–70	214 ± 10	175 ± 11*	202 ± 15	166 ± 9	207 ± 9	158 ± 6***
45–60	270 ± 11	231 ± 10**	266 ± 13	220 ± 11*	268 ± 8	194 ± 6***

Results are for duplicate tests in four rats before and after section of glossopharyngeal nerve (n.IX), in four rats before and after section of abdominal vagi (n.Xa) and in eight rats before and after section of both of these nerves. Significant effects of denervation are shown: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

The effect of sectioning n.Xa on the restored response to hypoxia

Hypoxic responses were tested in a further series of four rats 28 days after bilateral c.s.n. section. Bilateral n.Xa section was then performed and the animals restudied awake, 1 day later. n.Xa section eliminated the restored response of $\dot{V}_E$ in moderate hypoxia and reduced the residual response in severe hypoxia. V_T no longer increased at P_{I,O_2} 150–66 mmHg but still increased at P_{I,O_2} 53 mmHg, to a mean level of 2·39 ml compared with 1·91 ml in air. The frequency response at P_{I,O_2} 53 mmHg was reduced from a mean of 106 before to 92 after nerve section ($P < 0·05$). Details are shown in Fig. 2 and Table 1.

The effect of combined sectioning of n.IX and n.Xa on the restored response to hypoxia

The eight rats described above with bilateral c.s.n. section (29 days) and either n.IX or n.Xa section (1 day) were subjected to section of the remaining intact nerves (n.Xa or n.IX) and restudied, awake, 1 day later. Additional section of n.Xa after n.IX and c.s.n. section produced even lower values for frequency in severe hypoxia. n.IX section after n.Xa and c.s.n. section further reduced the frequency response at all levels of hypoxia. The sequence of n.IX and n.Xa sectioning did not affect the final response. Hypoxic responses of the eight rats with section of c.s.n. (30 days) n.IX (1 or 2 days) and n.Xa (1 or 2 days) are illustrated in Fig. 2 and detailed analyses are given in Table 1. In these animals $\dot{V}_E$ did not increase in mild hypoxia, was depressed in moderate hypoxia and increased in severe hypoxia. Frequency responses at P_{I,O_2} 105–52 mmHg were all significantly less than restored values ($P < 0·01$ to $< 0·001$) and at P_{I,O_2} 105–73 mmHg were below values in air. In severe hypoxia, P_{I,O_2} 52 mmHg, frequency increased to a mean level (81 min^{-1}) well below the restored level (106 min^{-1}). The V_T response, compared with results 28 days after c.s.n. section was reduced in air (mean value 1·97 ml before, 1·77 ml after, n.IX and n.Xa section, $P < 0·01$) and in mild and moderate hypoxia ($P < 0·01$ to $< 0·001$) but remained high in severe hypoxia (mean value at P_{I,O_2} 52 mmHg 2·52 ml before, 2·40 ml after n.IX and n.Xa section, the difference not statistically significant).

Comparison of the contribution of inputs in n.IX, n.Xa and a.d.n. to hypoxic responses 1 and 28 days after c.s.n. section

To answer whether the effects of the secondary inputs of the glossopharyngeal, abdominal vagal and aortic depressor nerves increased with time following carotid body denervation, a further series of experiments was undertaken. Bilateral c.s.n. section was performed on sixteen rats. In eight, hypoxic responses were tested after 1 day; the animals were then subjected to bilateral section of n.IX, n.Xa and a.d.n., done in a single procedure; and were retested, awake, 1 day later. These tests showed the contribution of the secondary chemoreceptors immediately after carotid body denervation. In the other eight, hypoxic responses were tested after 28 days; then bilateral section of n.IX, n.Xa and a.d.n. was performed, and the animals were restudied 1 day later to show the contribution of the secondary chemoreceptors to the restored hypoxic response.

In air and at all levels of hypoxia section of n.IX, n.Xa and a.d.n. produced greater reductions of $\dot{V}_E$ 28 days after c.s.n. section than 1 day afterwards (Fig. 3). Since initial

values of f, V_T, and $\dot{V}_E$ were higher 28 days after c.s.n. section than 1 day afterwards, in keeping with the restoration demonstrated in the first series of experiments, changes were calculated as percentages of the values found during air breathing in each case. The increased effect of denervations at 28 days remained apparent. It resulted mostly from increased changes of frequency.

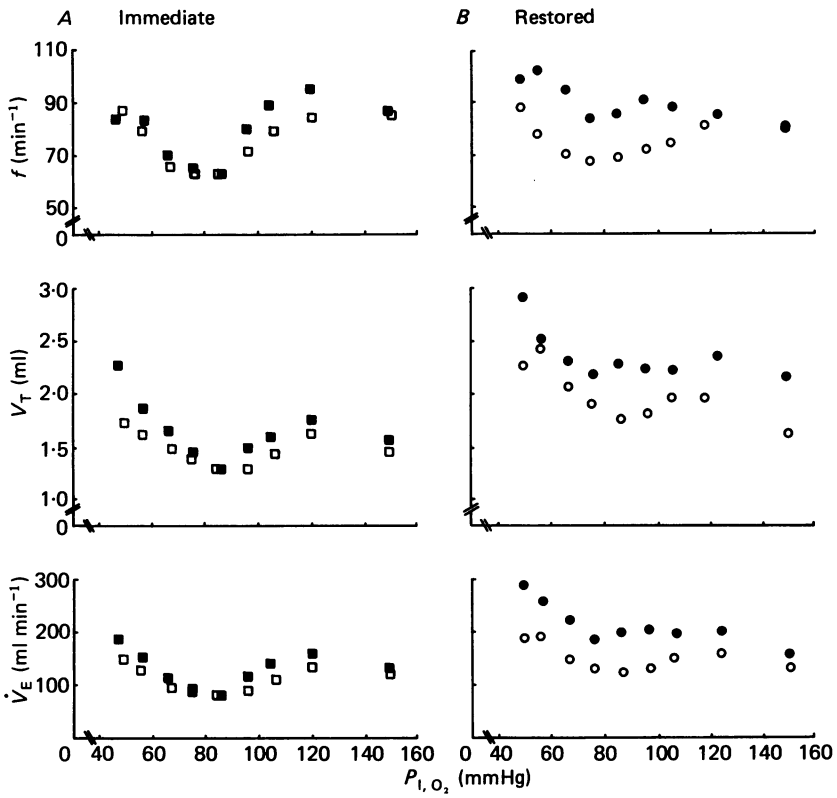


Fig. 3. The effects of denervation of secondary chemoreceptors on the response to hypoxia (*A*) immediately after carotid body denervation and (*B*) after restoration of the response. Left panel, values of f, V_T and $\dot{V}_E$ 1 day after bilateral section of c.s.n. (■); and 2 days after c.s.n. section and 1 day after section of n.IX, n.Xa and the aortic depressor nerve (□). Right panel, f, V_T and $\dot{V}_E$ 28 days after bilateral section of c.s.n. (●); and 29 days after c.s.n. section and 1 day after section of n.IX, n.Xa and a.d.n. (○) in eight rats. S.E. of means are included within area of symbols.

Residual hypoxic response after denervations. Contribution of a.d.n.

The data in Fig. 3 show the existence of residual ventilatory responses to hypoxia after all nerve sectioning procedures. Residual responses 29 days after c.s.n. section and 1 day after section of n.IX, n.Xa and a.d.n. (right-hand panel) were greater than responses measured 1 day after combined bilateral section of n.IX (which includes c.s.n.), n.Xa and a.d.n. (left-hand panel). Superimposition of results for $\dot{V}_E$ illustrate this finding (Fig. 4).

The effect of bilateral section of a.d.n. on restored hypoxic responses 29–30 days

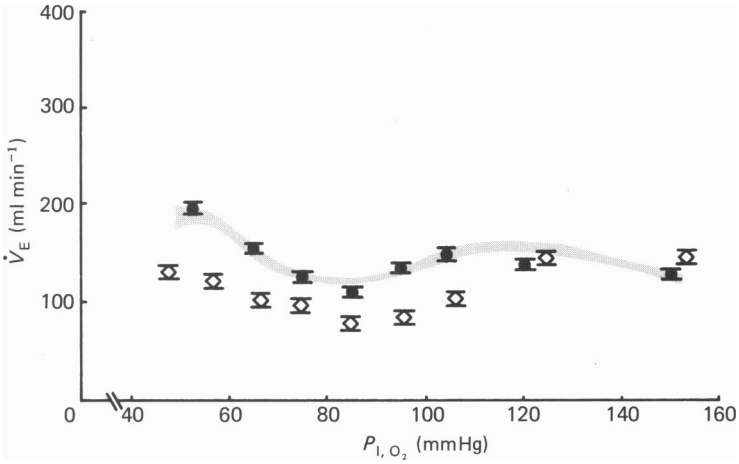


Fig. 4. Values for minute ventilation ($\dot{V}_E$) in Figs. 2 and 3 superimposed to show that acute peripheral chemo-denervation (section of n.IX, including c.s.n., n.Xa and a.d.n.) (⧆) leaves a lower residual ventilation than denervation of ancillary chemoreceptors (section of n.IX, n.Xa, a.d.n.) 28 days after c.s.n. section (shaded area, mean ± S.E. of mean) but the latter procedure without a.d.n. section (⧫) produces no significant change.

after bilateral section of c.s.n. and 1–2 days after bilateral section of n.IX and n.Xa can be seen by comparing data in Figs. 2 and 3. The close agreement of $\dot{V}_E$ (Fig. 4) indicates that the aortic bodies do not contribute to the residual hypoxic response.

DISCUSSION

In this series of experiments, acute denervation of peripheral chemoreceptors produced the general effects reported by Martin-Body *et al.* (1985), but the new results are of greater reliability because the animals served as their own controls whereas the previous study compared the results of tests in different groups of animals. The absence of a significant contribution of the aortic bodies to hypoxic respiration in the rat, illustrated by the data shown in Fig. 4 and by comparison of data in Fig. 3 with data published by Martin-Body *et al.* (1985), confirms neurophysiological observations of Sapru & Kreiger (1977) and anatomical observations of Barker *et al.* (1980).

The reported studies clearly demonstrate that after denervation of the carotid bodies by c.s.n. section there is a substantial though incomplete restoration of the ventilatory response to hypoxia. The extent of restoration shown in Fig. 1 is approximately 55% of the normal response. Restoration occurs within 3 weeks, is generally dependent on responses of respiratory frequency rather than tidal volume, and results partly from enhancement of effects of the inputs of glossopharyngeal and abdominal vagal nerves and partly from an effect of unidentified, possibly central, origin.

This study has demonstrated changes in frequency and tidal volume over a limited range of the stimulus response curve that are much larger than the modest systematic

errors present in barometric plethysmography (Fleming, Levine, Goncalves & Woollard, 1983; Body, 1984). The uniformity of the respiratory variables seen in this study (Table 1) at least partly results from restraining the rats within the plethysmograph chamber; measurements on rats free to move about the chamber give substantially more variable values for both tidal volume and frequency (Bartlett & Tenney, 1970; Sinclair, St. John & Bartlett, 1985).

The repeated recordings require long periods of experimental observation. For this reason and for ethical reasons, animal numbers are kept to the minimum required to give statistically reliable results. The consistency of observations between the different series of experiments represents important supporting evidence, however, for the significance of observed changes. The first series of four rats showed responses in the intact state similar to those in the thirty-four control studies previously reported (Martin-Body *et al.* 1985). Acute c.s.n. section produced the same results as previously reported. The restored hypoxic responses shown for the four animals (Fig. 1, 17–192 days) were also seen in the eight animals tested in the second and third series (Fig. 2) prior to additional nerve sectioning and were again illustrated, dramatically, in the final series of experiments. Here, two series of eight rats were tested 1 and 28 days after c.s.n. section (Fig. 3). Thus, while the serial effects at multiple intervals after denervation were tested in only four animals, the restoration of hypoxic responses was demonstrated in a total of twenty animals. Similarly the data on the role of the glossopharyngeal nerve in restoration at 28 days (Fig. 2) is supported by the data on eight rats shown in Fig. 3, right-hand panel.

In the absence of sham-operated controls, a question arises as to whether acute effects may be attributed to the anaesthetic regime, or cervical or abdominal surgery. The anaesthetic regime involved the application of minimal levels of halothane for generally short intervals. Although none of our tests were carried out on the day of anaesthesia, we have in other circumstances found normal hypoxic sensitivity within 3–5 h of comparable procedures. The modest response to acute bilateral abdominal vagotomy (Martin-Body *et al.* 1985) and to vagotomy 28 days after c.s.n. section (Fig. 2 middle panel) demonstrate that the surgical procedures can have little effect.

The value of making physiological studies on awake animals is self-evident but one technical disadvantage is the impracticability of monitoring arterial gas levels. This meant that hypoxic respiration was modified by varying levels of hypocapnia. Dead space effects would cause hypocapnic depression of response in animals with lower respiratory frequencies; but only small differences could be explained on this basis.

The study of recovery over a period of 6 months required consideration of age-dependent increases of tidal volume (Martin-Body & Sinclair, 1985). When normoxic respiration is associated with larger tidal volumes, proportional increases during hypoxic tests are represented by larger absolute increases of volume. Such increases, seen in serial studies on ageing rats, have previously been interpreted as evidence of complete restoration to the hypoxic response measured before carotid body denervation (Breslav & Konza, 1975). When responses are expressed as percentages of values obtained in normoxia, it is clear that restoration is incomplete.

Comparable studies of the extent and course of recovery have not been reported. Breslav & Konza (1975) reported restoration in the rat 1 month after denervation. A significant recovery of hypoxic sensitivity occurs in the cat at 30–43 days (Smith & Mills, 1980) and in ponies at 9 weeks (Bisgard *et al.* 1980). Ventilatory response

to the inhalation of 12% O_2 was absent in human subjects 2 weeks to 8 years after bilateral carotid body resection (Lugliani, Whipp, Seard & Wasserman, 1971); but restoration was seen, when specifically tested for, in studies made more than 23 years after denervation (Honda, Watanabe, Hashizume, Satomura, Hata, Sakakibara & Severinghaus, 1979).

Restoration of the hypoxic response

The significant elimination of restored hypoxic responses by sectioning of the glossopharyngeal nerves shows that compensation for loss of c.s.n. input depends on tissue supplied by the glossopharyngeal nerve. Our finding that the main role of abdominal vagal inputs in the restored hypoxic response lies in maintenance of frequency and minute ventilation at the lowest P_{I,O_2} levels testable further establishes a physiological role for the abdominal glomus tissue identified by Hollinshead (1946) and Andrews, Deane, Howe & Orbach (1972) and shown to hypertrophy in chronic hypoxia (Barker, Castro, Howe & Pack, 1984). Consistent with our findings on respiratory patterns, electrophysiological studies (Howe, Pack & Wise, 1981) show that the vagal chemosensory input is maximal in severe hypoxia.

It is improbable that restoration results from regeneration of carotid bodies or of carotid sinus nerve endings (Mitchell, Sinha & McDonald, 1972; Kienecker, Knoche & Bingman, 1978) since significant recovery occurred 3–17 days after extensive surgical separation of c.s.n. endings, adequate contact between nerve fibres and arterial blood is unlikely to be achieved, carotid body cells are required for chemoreception in regenerating fibres (Zapata, Hess & Eyzaguirre, 1969) and repetition of the destructive surgery does not reverse restoration (Breslav & Konza, 1975). It is more probable that in the rat, like the cat, glomus tissue along the subclavian arteries (Palkama & Hopsu, 1965) and carotid arteries (Matsuura, 1973) contributes an input in hypoxia of which the effects are enhanced after denervation of other chemoreceptor tissue.

The enhanced effects of subsidiary peripheral chemoreceptors might result from hypertrophy of the tissues. An alternative explanation is that synaptic reorganization occurs in the central nervous system, as occurs after interruption of sensory nerves in somatic reflex pathways (Gallego, Kuno, Nunez & Snider, 1979). Provisional evidence of synaptic degeneration led Majumdar *et al.* (1982) to propose that restored chemoreceptor responses resulted from an interaction between degenerative atrophy and regenerative proliferation of central projections of the interrupted sensory pathway and reactive synaptogenesis of converging projections of the uninterrupted pathway.

Restored responses were notably dependent on increases of frequency rather than tidal volume. The exception was the increase of tidal volume to levels greater than those of controls in c.s.n. sectioned rats subjected to mild hypoxia. This response was apparently not of singular origin, however, since it was eliminated by section of either glossopharyngeal or abdominal vagal nerves.

The residual response after peripheral chemodenervation

An especially interesting result in these experiments was that 28 days after c.s.n. section the residual ventilatory response to severe hypoxia after section of glossopharyngeal, abdominal vagal and aortic depressor nerves was more pronounced

than in acute experiments. In the circumstances, all known peripheral chemoreceptors are denervated. We have argued (Martin-Body *et al.* 1985) that the residual response is central in origin and has classically been overlooked because so few studies have been undertaken on the awake animal, whereas anaesthesia converts respiratory stimulation to a depression. It is however possible that the residual response results from the input from an unidentified peripheral chemoreceptor not served by the nerves sectioned. It is also theoretically possible that acute c.s.n. section lowers central excitability to a level where hypoxic stimulation is ineffective and that the increase of the residual response in longer term studies results from a return towards normal of the input–output relationships of respiratory regulation (Eldridge, Gill-Kumar & Millhorn, 1981). This explanation may in particular account for the observation that the depression of respiration seen in these studies 3 days after chemo-denervation was less than that seen 1 day afterwards (Martin-Body *et al.* 1985).

In summary, the experiments demonstrated considerable restoration of hypoxic ventilatory responses after carotid body denervation, an important observation in terms of the assumption often made in physiological experiments that peripheral chemo-denervation is permanent. The restoration depended largely on enhancement of the effects of ancillary glomus tissue served by glossopharyngeal nerves, and also on tissue supplied by the abdominal vagi. Restoration still present after denervation of all known peripheral chemoreceptors may result either from inputs from further unidentified peripheral chemoreceptors or from a central mechanism serving respiratory stimulation in hypoxia in the awake animal.

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