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THE CARDIOVASCULAR RESPONSES TO STIMULATION OF THE CAROTID BODY CHEMORECEPTORS IN THE DOG

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As far as we are aware there have been no previous reports in the literature concerning the effect of reflexes from the carotid body chemoreceptors on cardiac output. We have recently carried out such an investigation in the anaesthetized dog and in the first part of the paper the results obtained both in animals breathing spontaneously and ventilated artificially are described. Some of the mechanisms responsible for the observed cardiovascular responses in the two types of preparation were also studied, and these are described in subsequent sections of the paper. Some of our results have been reported briefly elsewhere (Daly & Scott, 1959, 1963).

METHODS

Dogs varying in weight from 13·8 to 44·0 kg were anaesthetized with either a mixture of chloralose (Roche Products Ltd., 0·05 g/kg) and urethane (British Drug Houses Ltd., 0·5 mg/kg) intravenously after premedication with morphine hydrochloride (2 mg/kg subcutaneously), or pentobarbitone sodium (Nembutal, Abbott Laboratories, Ltd., 40 mg/kg I.V.). Systemic blood pressure was recorded from a femoral artery with a Hürthle manometer. Heart rate was counted over a period of ½ min from the blood pressure trace taken on a fast-moving paper. The animals breathed room air and the tidal volume was recorded on the kymograph by means of a balanced spirometer using the method described by Donald & Christie (1949). All gas volumes are expressed at A.T.P.S.

In some experiments artificial respiration was applied by means of a Starling 'Ideal' pump. In those experiments in which a bilateral open pneumothorax was made, the lungs were allowed to collapse passively against a resistance of 2–3 cm H_2O. Spontaneous respiratory efforts were prevented by decamethonium iodide (0·25 mg/kg I.V., Light and Co. Ltd.), anaesthesia being maintained in these experiments by regular injections of the anaesthetic, the dose having been determined previously in a series of experiments on spontaneously breathing dogs.

A double-lumen cannula was inserted through the right external jugular vein into the right atrium. The right atrial pressure was measured by means of a vertical saline manometer, the open end of which was connected to a small volume recorder. The zero reference point was taken from the tip of the cannula established post mortem.

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Perfusion of the carotid bodies

Both carotid bifurcation regions were isolated from the circulation and perfused with oxygenated blood from a carotid artery of a donor animal breathing room air. The carotid body chemoreceptors were stimulated by changing the perfusate from oxygenated to hypoxic blood, brought about by giving the donor dog 5 % O_2 in N_2 to breathe. The carotid sinus pressure, recorded with a mercury manometer, was maintained at the same level as the recipient dog's systemic blood pressure by the method described by Daly & Scott (1958).

Measurement of cardiac output

Cardiac output was determined by the dye-dilution method (Moore, Kinsman, Hamilton & Spurling, 1929). The dye (T–1824, Evans Blue, British Drug Houses, Ltd.), in doses of 3·6–6·55 mg according to the weight of the animal, was injected into the right atrium through one lumen of the double-lumen catheter inserted through the right external jugular vein. The exact amount of dye injected was determined by taking the difference in the weight of each syringe before and after injection. Blood from the arch of the aorta was continuously sampled via a catheter inserted through the right common carotid artery, and collected in a series of small tubes arranged on the periphery of a moving kymograph drum. An equal volume of blood was transfused at the end of each sampling period. The concentration of dye in each sample of blood was estimated at a wave-length of 600 mμ (Ilford 607 filter) by means of a Hilger–Spekker absorptiometer. A calibration curve was obtained at the beginning of each experiment, using the blood of the recipient animal.

It was found that duplicate determinations of cardiac output under constant conditions in four dogs did not differ by more than 4 %. The absolute accuracy of the method has not, however, been determined.

The 'central' blood volume was calculated from the dye-dilution curve according to Hamilton, Moore, Kinsman & Spurling (1932).

Total peripheral (systemic) vascular resistance (T.P.R.) was calculated thus:

$$\text{T.P.R.} = \frac{\text{Systemic blood pressure} - \text{right atrial pressure (mm Hg)}}{\text{Cardiac output (ml./min)}}.$$

In some experiments made on preparations with open chest, changes in cardiac output were inferred from changes in blood flow to one lobe of the lungs (Hurlimann & Wiggers, 1953; Daly, 1957). Blood flow to the left diaphragmatic lobe was measured and recorded continuously by means of a Shipley & Wilson (1951) type of rotameter in which were incorporated the modifications of Bell (1954). The calibration of the rotameter and measurements of mean left and right atrial pressures and mean pulmonary arterial pressure were made as described previously (Daly, 1957).

Preparation of the animals for subsequent denervation of the lungs

In six animals both thoracic vagosympathetic nerves were divided between the origin of the cardiac and the pulmonary branches during the acute experiment without opening the thorax. This was done by placing snares on each thoracic vagosympathetic nerve at a previous operation, the procedure being the same as that described by Daly & Scott (1958).

Blood analyses

Oxygen and carbon dioxide contents of blood were determined by the manometric method of Van Slyke & Neill (1924). The haemoglobin concentration was determined by Stadie's (1920) cyanhaemoglobin method, as modified by Wu (1922). Oxygen capacity was calculated from the haemoglobin concentration on the assumption that 1 g haemoglobin combines with 1·34 ml. O_2.

Estimations of blood pH were made in duplicate at room temperature, a McInnes–Belcher glass electrode and a pH meter (Model C33B, Electronic Instruments, Ltd.) being

used in conjunction with a Vibron electrometer (Model 33B, Electronic Instruments, Ltd.). The instrument was standardized before every determination by means of phosphate buffers of pH 7·40–7·60 at 20° C. The pH of the blood was measured at room temperature (18–22° C) and was converted to the corresponding value at 37° C using the temperature coefficient of Rosenthal (1948). The effect of the presence of cells on the estimation of plasma pH was allowed for by adding 0·01 to the measured pH of the blood (Severinghaus, Stupfel & Bradley, 1956*a*).

The carbon dioxide tension was calculated from the observed values for carbon dioxide content, pH and percentage oxygen saturation using the formula

$$P_{CO_2} = \frac{\text{Serum } CO_2}{S(10^{pH-pK'}+1)}$$

(Severinghaus *et al.* 1956*a*). The serum CO_2 content (mM) was calculated by multiplying the value for whole blood CO_2 content by the factor '*f*' obtained from the nomogram of Van Slyke & Sendroy (1928). The solubility factor *S* was read from the table of Severinghaus *et al.* (1956*a*), and the value for pK' from the nomogram of Severinghaus, Stupfel & Bradley (1956*b*).

In all experiments heparin ('Liquemin', Roche Products, Ltd.) was given in doses of 7–22 mg/kg to render the blood incoagulable.

Experimental procedure. A control measurement of cardiac output was made during perfusion of the carotid bodies with oxygenated blood, the donor dog breathing room air. The chemoreceptors were then stimulated by hypoxic blood, by substituting, in the donor animal, 5 % O_2 in N_2 for room air. When a steady state had been reached in the recipient animal, as indicated by the levels of the tidal volume, respiratory frequency, heart rate and blood pressure, another determination of cardiac output was made. Finally, after re-establishing oxygenated blood perfusion of the carotid bodies a second control measurement of cardiac output was made. The control values quoted are the mean of the two measurements made before and after the tests of chemoreceptor stimulation. In some experiments the P_{CO_2} of the arterial blood was maintained constant, or very nearly so, during tests of chemoreceptor stimulation in the following way: before stimulation of the carotid bodies was begun, a measurement of cardiac output was made with the recipient animal breathing room air. When hypoxic blood perfusion of the recipient animal's carotid bodies was established a gas mixture containing 4–7 % CO_2, 21 % O_2 in N_2 was substituted for room air. At the end of the test of chemoreceptor stimulation room air breathing was re-established before the final control measurements were made. The constancy of the arterial blood P_{CO_2} was checked by analysis of the blood.

RESULTS

The results of twenty-three tests of stimulation of the carotid body chemoreceptors by hypoxic blood carried out in nineteen experiments in dogs with natural respiration are shown in Table 1.

Respiratory minute volume. There invariably occurred an increase in respiratory minute volume of 46–493 % of the control value (mean 219 %) for the experiments under chloralose-urethane anaesthesia. Both the tidal volume and respiratory frequency were increased (Fig. 1). In two tests in one experiment under Nembutal there was a twelve- and a fifteenfold increase, respectively (Table 1).

Heart rate. As found previously (Daly & Scott, 1958) the responses were variable (Table 1). An increase in rate occurred in twelve tests in ten

TABLE 1. The effects of stimulation of the carotid body chemoreceptors by hypoxic blood in dogs spontaneously breathing room air. The results have been grouped arbitrarily according to whether the cardiac output increased (*A*), decreased (*B*), or did not change (*C*). C and E are the control and experimental states respectively

Expt. no.	Dog wt. (kg)	Heart rate (beats/min)		Blood pressure (mm Hg)		Cardiac output (l./min)		'Central' blood volume (l.)		Total peripheral vascular resistance (mm Hg/ml./min × 100)		Respiratory minute volume (l./min)	
		C	E	C	E	C	E	C	E	C	E	C	E
						Chloralose–urethane anaesthesia							
A 36	22·0	96	108	125	125	2·91	3·36	0·33	0·34	4·29	3·72	—	—
40*a*	19·5	106	144	125	115	2·50	3·10	0·54	0·45	5·03	3·78	3·32	10·08
40*b*	19·5	102	123	125	115	2·51	2·67	0·54	0·40	5·00	4·35	3·02	9·10
42*a*	35·0	105	108	123	120	3·42	4·52	0·60	0·67	3·58	2·66	3·11	14·50
42*b*	35·0	105	117	123	115	3·50	4·68	0·64	0·68	3·51	2·46	3·50	11·60
44	18·7	95	135	105	100	1·81	2·16	0·26	0·25	5·80	4·63	1·94	6·05
46*a*	22·5	110	105	110	108	2·46	3·17	0·53	0·59	4·48	3·42	1·86	5·90
46*b*	22·5	111	106	110	100	2·48	3·12	0·54	0·62	4·45	3·23	2·70	10·60
47	26·1	84	90	120	110	2·95	3·26	0·63	0·60	4·07	3·38	2·48	3·63
53	24·9	81	96	135	125	2·60	3·01	0·55	0·55	5·19	4·15	3·23	10·00
56	27·6	126	180	103	85	3·01	3·42	0·53	0·45	3·42	2·49	4·93	29·20
57	44·0	78	93	113	110	3·37	4·62	0·74	0·73	3·35	2·43	6·81	34·00
58	33·9	135	138	105	90	2·21	2·85	0·45	0·43	4·75	3·15	5·22	27·10
61	20·0	165	129	112	120	3·23	3·95	0·33	0·36	3·46	3·02	7·78	17·60
62	21·6	150	138	125	120	2·56	3·00	0·31	0·40	4·87	4·00	3·87	11·20
65	17·8	96	108	130	115	2·18	2·46	0·32	0·29	5·96	4·67	3·62	11·78
B 48	16·5	100	81	137	125	2·62	2·32	0·42	0·43	5·24	5·40	3·06	6·32
59	24·3	107	96	130	125	3·80	3·27	0·39	0·40	3·42	3·82	5·78	10·16
64	23·7	126	126	130	125	2·74	2·54	0·35	0·36	4·74	4·92	2·76	6·00
C 60	24·8	99	98	115	110	4·06	4·08	0·51	0·54	2·83	2·69	5·35	10·05
63	23·3	144	141	125	120	3·57	3·60	0·45	0·44	3·50	3·34	5·30	10·88
						Nembutal anaesthesia							
101 *a*	25·5	170	210	135	120	2·84	5·35	0·50	0·55	4·75	2·24	3·92	51·10
101*b*	25·5	177	200	124	110	2·89	4·44	0·51	0·54	4·30	2·48	3·06	51·20

experiments (see Fig. 1), a decrease in seven tests in six experiments, and no change in rate in one test in each of two experiments.

Blood pressure. In nineteen tests the systemic blood pressure fell during stimulation of the carotid bodies, the maximum response being 17 mm Hg. In the two remaining tests it increased by 8 mm Hg in one, and did not change in the other.

Cardiac output. The effects on cardiac output are given in detail in Table 1. All experiments carried out under chloralose-urethane anaesthesia have been grouped arbitrarily into three categories, according to whether the output increased, decreased or did not change. It will be noted in Table 1 that stimulation of the carotid bodies caused an increase in cardiac output in the majority of experiments. An example of this type of response is shown in Fig. 1.

When the mean values for cardiac output are calculated for each group and expressed as cardiac index, it is found that in sixteen tests in thirteen experiments the cardiac output increased from a mean value of 2·99 l./min/m^2 to a mean value of 3·62 l./min/m^2, the mean increase being 0·63 l./min/m^2 (range 0·20–1·02). In three tests in three experiments the mean value for the cardiac output decreased from 3·70 to 3·29 l./min/m^2 (mean decrease 0·42 l./min/m^2; range 0·22–0·59). In two tests in two experiments the cardiac output (4·30 l./min/m^2) was not affected by chemoreceptor stimulation.

Two tests of stimulation of the carotid bodies were made in one dog under Nembutal anaesthesia (Table 1), and an increase in cardiac output of 2·71 and 1·70 l./min/m^2 respectively was observed.

Total peripheral vascular resistance. In all thirteen experiments in which carotid body stimulation caused an increase in cardiac output (sixteen tests, Table 1 *A*), the total peripheral vascular resistance decreased by an average of 22·2 % (range 13–34 %). In one experiment (No. 59, Table 1 *B*) the resistance increased 12 %, whereas in the remaining four (Nos. 48, 60, 63 and 64 in Table 1 *B* and *C*) it did not change significantly. In the experiment under Nembutal anaesthesia the total peripheral vascular resistance fell by 53 and 42 %, respectively, in the two tests.

'Central' blood volume. As shown in Table 1, in those experiments in which stimulation of the carotid bodies caused tachycardia and an increase in cardiac output, the 'central' blood volume either decreased or did not change. The two exceptions were experiments Nos. 42 and 101, in which the volume increased. In all experiments in which slowing of the heart was a prominent feature an increase in 'central' blood volume occurred.

All the above responses to hypoxic stimulation of the carotid bodies were abolished by cutting the carotid sinus nerves, and are therefore reflex in nature.

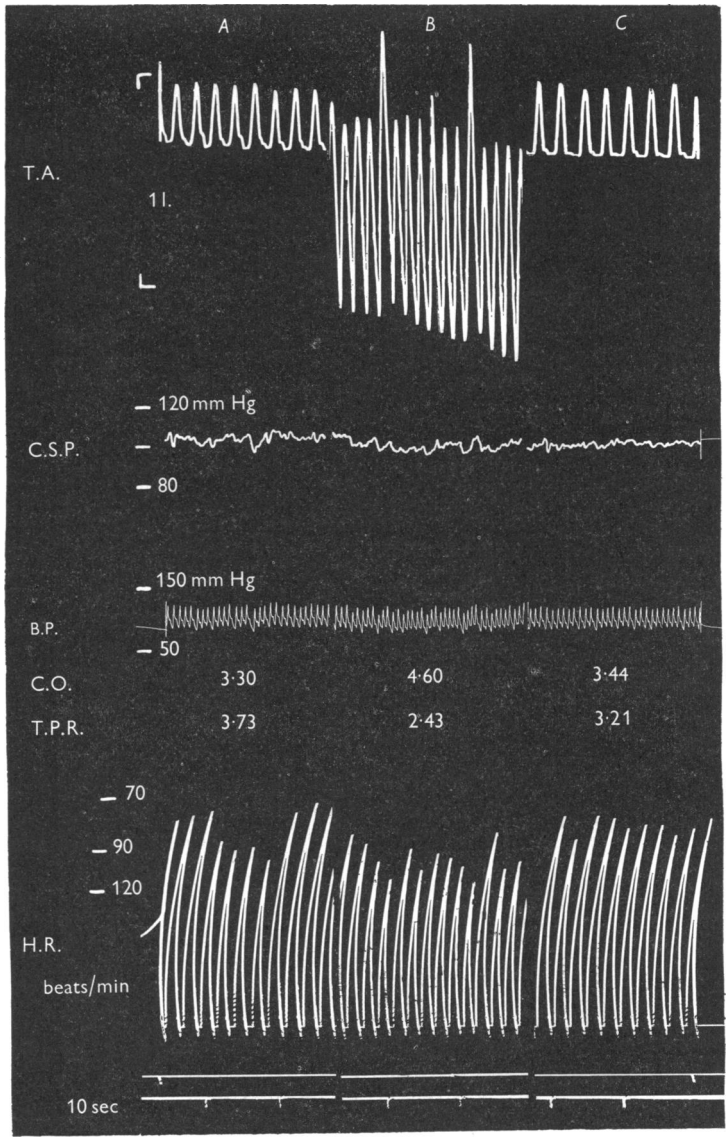

Fig. 1. Dog (*m*) 44·0 kg. Morphine–chloralose–urethane. Spontaneous respiration. *A* and *C* are control records taken before and after stimulation of the carotid body chemoreceptors (*B*) respectively. The values below the blood pressure trace are those for cardiac output (l./min) and total peripheral vascular resistance (mm Hg/ml./min × 100). In this and in subsequent figures: R.M.V., respiratory minute volume; T.A., tidal air volume; L.A.P., left atrial pressure; R.A.P., right atrial pressure; P.L.F., pulmonary lobar blood flow (left diaphragmatic lobe); P.A.P., pulmonary arterial pressure; C.S.P., carotid sinus pressure; B.P., systemic blood pressure; C.O., cardiac output; T.P.R., total peripheral vascular resistance; H.R., heart rate. Time marker, 10 sec.

These results show that, in the dog breathing spontaneously, carotid body chemoreceptor stimulation by hypoxic blood usually causes tachycardia, an increase in cardiac output, a small fall in systemic blood pressure and a decrease in total peripheral vascular resistance. Evidence has been presented elsewhere (Daly & Scott, 1958, 1962, 1963) that in the spontaneously breathing dog the cardio-acceleration and vasodilatation occurring in response to stimulation of the carotid bodies are not due to primary cardiac and vascular reflexes from these chemoreceptors, but are secondary to the concomitant reflex hyperpnoea. When the frequency and depth of breathing are maintained constant, stimulation of the chemoreceptors invariably caused bradycardia and vasoconstriction. These findings receive further support in experiments reported in the next section of this paper.

Dogs ventilated artificially

In experiments carried out on dogs with open chest and ventilated artificially, changes in cardiac output were inferred from changes in blood flow to one lobe of the lung, measured with a rotameter flowmeter. That this inference is a valid one has been shown by Daly (1957), who demonstrated that, provided ventilation remained constant, changes in lobar blood flow reflected similar directional changes in total pulmonary blood flow.

It was found that stimulation of the carotid body chemoreceptors by hypoxic blood invariably caused a reduction in cardiac output (thirteen tests in seven experiments). The typical response is shown in Fig. 2. The systemic blood pressure increased or remained unchanged, despite the reduction in cardiac output. The total peripheral vascular resistance must, therefore, have increased. In two other tests systemic blood pressure fell and it is not possible, therefore, to determine from qualitative measurements of cardiac output whether or not there was any change in total peripheral vascular resistance. In all tests the heart slowed, as found previously in dogs ventilated artificially (see Daly & Scott, 1963), the pulmonary arterial pressure fell and the left and right atrial pressures increased (Fig. 2).

These results were confirmed in one experiment carried out on a dog with a closed chest and ventilated artificially. Cardiac output in this experiment was determined by the dye-dilution method. Carotid body chemoreceptor stimulation caused a reduction in cardiac output in two tests, from 1·40 to 1·16 l./min and from 1·57 to 1·38 l./min, respectively. Systemic blood pressure rose from 102 to 110 mm Hg in the first test, but remained unchanged at 100 mm Hg in the second. The calculated total peripheral vascular resistance increased by 30 % and 13·5 % respectively in two tests. This confirms the results of other workers, who found that

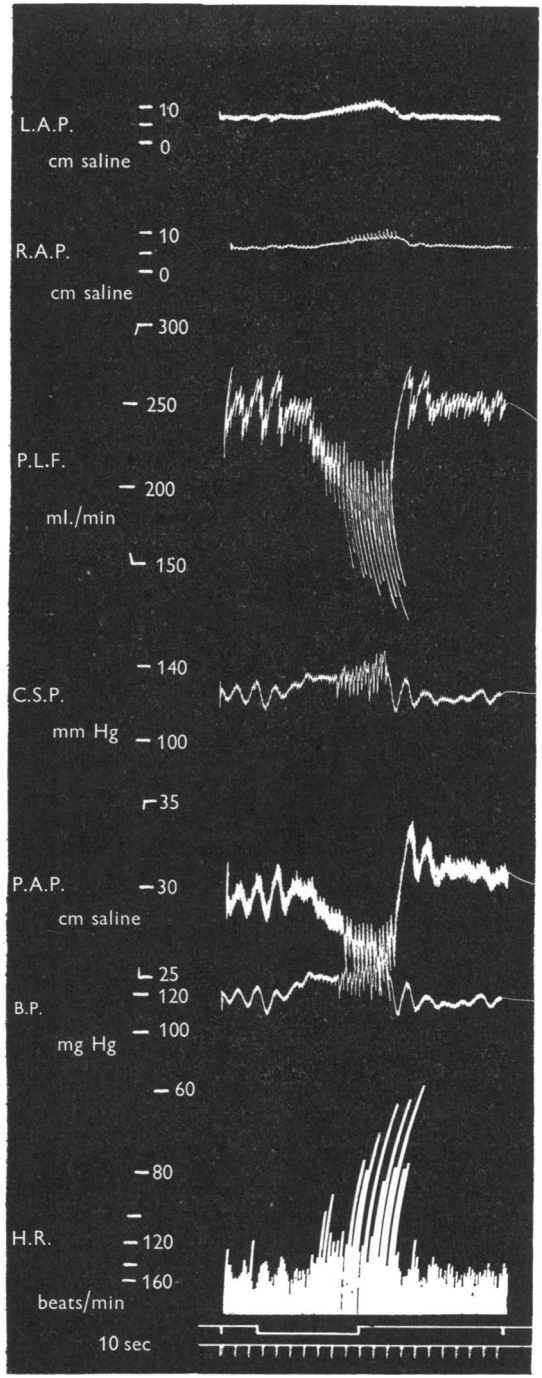

Fig. 2. Dog (*f*), 17·3 kg. Morphine–chloralose–urethane. Open chest. Positive-pressure ventilation. Measurement of blood flow to the left diaphragmatic lobe. During signal, stimulation of the carotid bodies by hypoxic blood.

carotid body stimulation, in dogs artificially ventilated, caused peripheral vasoconstriction (Bernthal, 1938; Daly & Daly, 1959; Daly & Scott, 1962).

In these experiments the diminution in cardiac output occurring on stimulation of the carotid bodies must be attributed largely to the reduction in heart rate. If the test is repeated after administration of atropine (one experiment) or after division of the cervical vagosympathetic nerves (two experiments), the bradycardia is considerably reduced (Daly & Scott, 1958, 1962, 1963; Downing, Remensnyder & Mitchell, 1962) and now an increase in cardiac output occurs, not a decrease. The results of these experiments are shown in Fig. 3. The reduction in pulmonary lobar blood flow is abolished by hexamethonium bromide (10 mg/kg intravenously; May and Baker, Ltd.).

These experiments show that in the dog with controlled ventilation stimulation of the carotid bodies causes bradycardia, a reduction in cardiac output and an increase in peripheral vascular resistance; that is, responses exactly opposite to those observed in the majority of experiments made on spontaneously-breathing dogs.

Daly & Scott (1958, 1962) presented evidence that one secondary mechanism by which carotid body stimulation causes tachycardia in the spontaneously breathing dog is an inflationary reflex from the lungs (see Anrep, Pascual & Rössler, 1936*a*, *b*), since the response is reversed after denervation of the lungs. In addition to a reflex from the lungs another mechanism which may be involved is a fall in arterial blood P_{CO_2} caused by the reflex increase in respiratory minute volume. The experiments now to be described were undertaken to gain further information on the mechanisms determining the directional changes in heart rate, cardiac output and total peripheral vascular resistance in response to chemoreceptor stimulation in the spontaneously breathing dog.

Denervation of the lungs

Five experiments were carried out on dogs spontaneously breathing room air, in which the carotid body chemoreceptors were stimulated before and after denervation of the lungs by division of the pulmonary branches of the thoracic vagosympathetic nerves without having to open the thorax (Daly & Scott, 1958). It was found that denervation of the lungs caused changes in the response of the cardiovascular system to stimulation of the carotid bodies which resulted in the primary cardiac and vascular effects, namely bradycardia and vasconstriction, becoming more apparent. The results are shown in Fig. 4 and may be summarized as follows:

(1) Denervation of the lungs reversed the cardio-accelerator response (Expts. Nos. 53 and 56) or enhanced the cardio-inhibitory response (Expt.

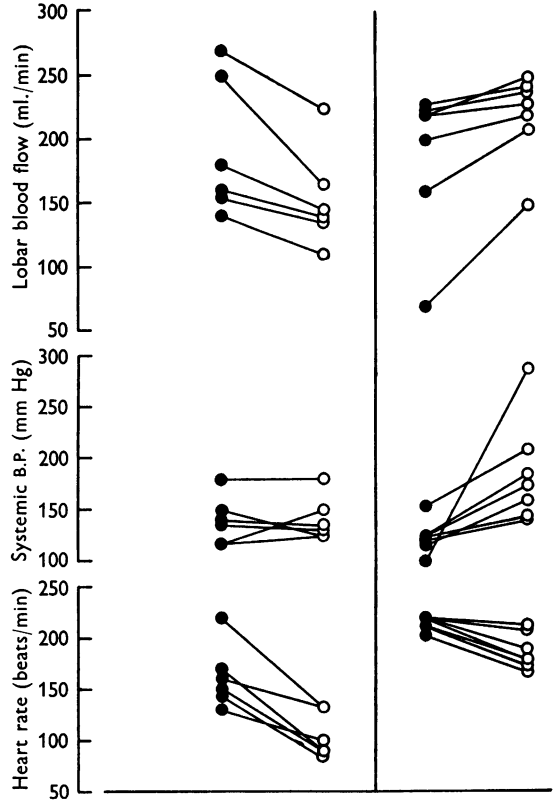

Fig. 3. The results of three experiments on dogs ventilated artificially, showing the values for pulmonary lobar blood flow, systemic blood pressure and heart rate in response to stimulation of the carotid bodies by hypoxic blood before and after division of the cervical vagosympathetic nerves (two experiments) and administration of atropine 3 mg (one experiment). Filled circles (●), control values during perfusion of the carotid bodies with oxygenated blood; open circles (○), values during stimulation of the chemoreceptors.

No. 61), confirming the previous observations of Daly & Scott (1958). In Expt. No. 62 the cardiac response was not affected by lung denervation, whereas a slight tachycardia occurred in Expt. No. 63.

(2) In three out of five experiments (Nos. 53, 56 and 61) there was a tendency for the primary vascular response to stimulation of the carotid bodies to become more apparent after denervation of the lungs. On the other hand the response was variable in the remaining two experiments (Nos. 62 and 63).

(3) The effects of lung denervation on the cardiac output response were small and variable. In four experiments there was an increase in cardiac

output on stimulation of the carotid bodies before denervation of the lungs; in one (Expt. No. 63) there was no significant change.

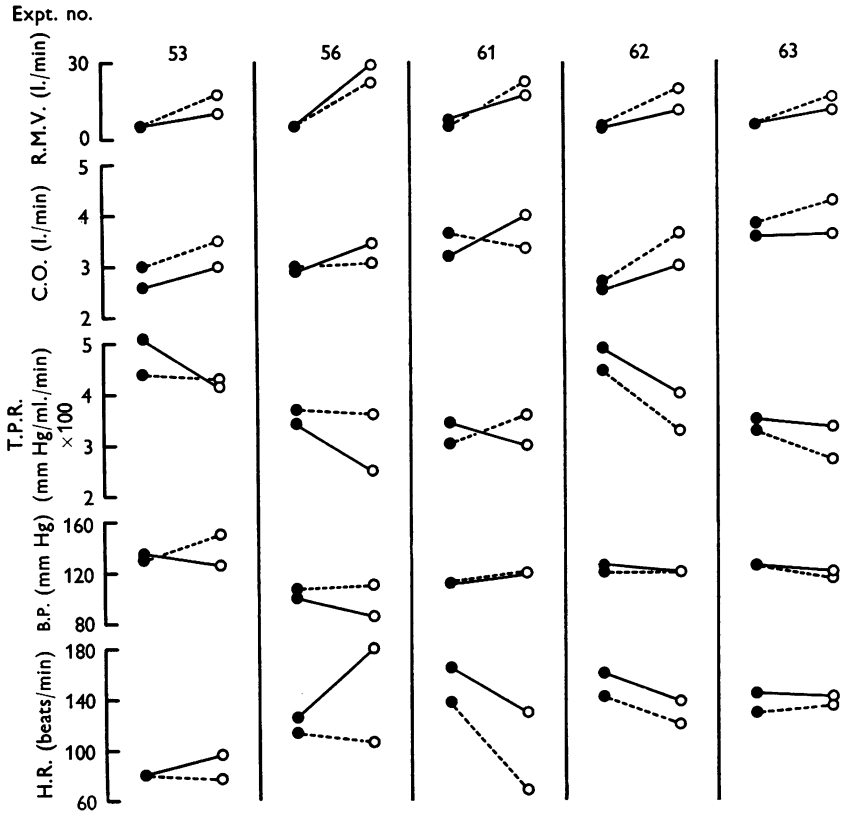

Fig. 4. The effects of stimulation of the carotid body chemoreceptors by hypoxic blood in five experiments, before (●—○) and after (●----○) denervation of the lungs. Filled circles (●), control values during perfusion of the carotid bodies with oxygenated blood: open circles (○), values during stimulation of the chemoreceptors.

Maintenance of constant arterial blood P_{CO_2}

Three experiments were carried out in which the carotid body chemoreceptors were stimulated by hypoxic blood, first while the animal was breathing room air, and secondly while it breathed a gas mixture containing 4–7 % CO_2 and 21 % O_2 in N_2 to maintain the arterial blood Pco_2 at its initial value.

The results obtained in these experiments are shown in Table 2 and can be summarized as follows:

(1) In two out of three experiments (Nos. 58 and 60) chemoreceptor stimulation during air breathing had no appreciable effect on heart rate.

When the tests were repeated while the arterial blood P_{CO_2} was maintained constant, a bradycardia occurred. In the third experiment (No. 65), the increase in heart rate was converted to a decrease.

(2) A reduction in total peripheral vascular resistance occurred on stimulation of the carotid bodies in all three experiments while room air was breathed. When the test was repeated while the arterial blood P_{CO_2} was maintained constant the response was reduced in size in one experiment (No. 58), abolished in the second (No. 60) and converted to an increase in vascular resistance in the third (No. 65).

(3) The increase in cardiac output which occurred in two out of the three experiments was abolished in one and converted to a fall in the other (No. 68) when the arterial blood P_{CO_2} was maintained constant. In the third experiment, in which no change in output occurred in the control test, chemoreceptor stimulation caused a fall when the test was repeated while maintaining the arterial blood P_{CO_2} constant (No. 60).

The results of these experiments show that when, during chemoreceptor stimulation, the arterial blood P_{CO_2} is prevented from falling, there is a tendency for the primary cardiac and vascular reflex responses to become more apparent. They strongly suggest, therefore, that the reduction in arterial blood P_{CO_2} which occurs in the spontaneously breathing dog is responsible, at least in part, for the observed cardiovascular responses.

Combined denervation of the lungs and maintenance of a constant arterial blood P_{CO_2}

The results obtained in three experiments are shown in Table 3. In Expt. No. 61 the initial test (*a*) of stimulation of the chemoreceptors, when the animal was breathing room air, caused bradycardia, an increase in cardiac output and a reduction in total periperal vascular resistance. After denervation of the lungs (*b*), chemoreceptor stimulation caused a more marked bradycardia, but now the responses of the cardiac output and total peripheral vascular resistance were reversed; the output decreased whereas the vascular resistance increased. When the test was repeated after denervation of the lungs and while the recipient animal's arterial blood P_{CO_2} was maintained constant during the period of chemoreceptor stimulation (*c*), there was a further enhancement of the bradycardia and of the reduction in cardiac output. The total peripheral vascular resistance increased still further. A similar response occurred in a second experiment (No. 62 in Table 3). Thus, in these two experiments the primary cardiovascular effects of chemoreceptor stimulation were unmasked by combined denervation of the lungs, and maintenance of a constant arterial blood P_{CO_2}.

TABLE 2. The effects of stimulation of carotid body chemoreceptors on the cardiovascular system during ventilation (*a*) with room air, and (*b*) with 4–7 % CO_2, 21 % O_2 in N_2. C control state; E, experimental state

Expt. no.	Dog wt. (kg.)	Heart rate (beats/min)		Systemic B.P. (mm Hg)		Cardiac output (l./min)		Peripheral vascular resistance (mm Hg/ml./ min × 100)		Respiratory minute volume (l./min)		Arterial blood P_{CO_2} (mm Hg)	
		C	E	C	E	C	E	C	E	C	E	C	E
58*a*	33·9	135	138	105	90	2·21	2·85	4·75	3·16	5·22	27·1	57	27
58*b*	33·9	141	124	107	100	2·42	2·43	4·42	4·10	5·84	35·7	57	56
60*a*	24·8	99	98	115	110	4·06	4·08	2·83	2·69	5·35	10·05	43	33
60*b*	24·8	92	88	117	112	4·28	4·03	2·74	2·78	5·68	13·0	43	41
65*a*	17·8	96	108	130	115	2·18	2·46	5·96	4·68	3·62	11·78	37	25
65*b*	17·8	104	87	127	125	2·73	2·48	4·65	5·05	4·99	18·2	37	40

TABLE 3. The effects of stimulation of the carotid body chemoreceptors on the cardiovascular system (*a*) before and (*b*) after denervation of the lungs; and (*c*) during maintenance of a constant arterial blood P_{CO_2} after lung denervation. C, control state; E, experimental state

Expt. no.	Dog wt. (kg)	Heart rate (beats/min)		Systemic B.P. (mm Hg)		Cardiac output (l./min)		Peripheral vascular resistance (mm Hg/ml./ min × 100)		Respiratory minute volume (l./min)		Arterial blood P_{CO_2} (mm Hg)	
		C	E	C	E	C	E	C	E	C	E	C	E
61*a*	20·0	165	129	112	120	3·23	3·95	3·47	3·02	7·78	17·6	—	—
61*b*	20·0	138	88	112	120	3·67	3·34	3·05	3·60	5·35	22·1	—	20
61*c*	20·0	138	62	105	115	3·01	2·12	3·50	5·43	8·55	27·6	38	45
62*a*	21·6	150	138	125	120	2·56	3·00	4·88	4·00	3·87	11·2	—	—
62*b*	21·6	132	120	120	120	2·70	3·67	4·44	3·27	3·46	18·9	46	26
62*c*	21·6	132	84	120	130	3·13	2·48	3·83	5·24	2·68	24·1	53	55
63*a*	23·25	144	141	125	120	3·57	3·60	3·50	3·34	5·30	10·9	—	—
63*b*	23·25	129	135	125	115	3·82	4·26	3·27	2·70	5·31	16·1	41	29
63*c*	23·55	144	120	125	115	3·45	3·63	3·63	3·17	4·86	16·0	44	45

In the third experiment of this type (Expt. No. 63) neither denervation of the lungs alone, nor combined lung denervation and a constant arterial blood P_{CO_2} was entirely effective in unmasking the primary cardiac and vascular reflex effects of chemoreceptor stimulation.

DISCUSSION

Our results have shown that, in the spontaneously breathing dog, stimulation of the carotid body chemoreceptors by hypoxic blood causes an increase in respiratory minute volume and variable effects on the cardiovascular system. Tachycardia, an increase in cardiac output, and a reduction in total peripheral vascular resistance are, however, the responses which predominate. In this connexion variable effects on heart rate (Bernthal, Greene & Revzin, 1951; Daly & Scott, 1958, 1962) and on vascular resistance in perfused limbs and splanchnic vascular bed (Daly & Scott, 1962) have been reported previously.

A more detailed examination of these cardiovascular effects revealed that they were determined to a large extent by the concomitant reflex hyperventilation. The evidence for this was obtained in experiments in which pulmonary ventilation during chemoreceptor stimulation was maintained constant and at the same time spontaneous respiratory efforts were prevented by decamethonium. This neuromuscular blocking drug does not appreciably affect either carotid chemoreceptor or baroreceptor reflexes in the dog (Daly & Scott, 1958; M. de B. Daly and J. L. Hazzledine, unpublished observations). Under these conditions it was found that stimulation of the carotid bodies invariably caused bradycardia, a reduction in cardiac output and an increase in total peripheral vascular resistance.

These results fall into line with those reported previously, in so far as it has been shown that stimulation of the carotid bodies by hypoxic blood in dogs with controlled ventilation causes bradycardia (Bernthal *et al.* 1951; Daly & Daly, 1957, 1959; Daly & Scott, 1958, 1962; Downing *et al.* 1962), and peripheral vasoconstriction (Bernthal, 1938; Daly & Daly, 1959; Daly & Scott, 1962). These responses represent the primary reflex cardiac and vascular effects from the carotid bodies (Daly & Scott, 1962, 1963).

Changes in cardiac output

The mechanisms governing the changes in cardiac output are more complex and deserve further consideration. On the one hand the increase in output occurring on excitation of the carotid bodies in dogs breathing naturally in probably due, at least in part, to increased aspiration of blood into the thorax by virtue of the reflex stimulation of respiration, and to vasodilatation, principally in skeletal muscle and in the splanchnic vascular

bed (Daly & Scott, 1962), resulting in an increase in *vis a tergo*. In addition there may also be a positive inotropic effect on the ventricles accompanying acceleration of the heart. In this connexion Monroe, French & Whittenberger (1960) found no consistent change in myocardial contractility during hypocapnia. In their experiments, however, the rate and depth of breathing was constant and an inflationary reflex from the lungs, which plays an important role in determining the cardiovascular responses to hyperventilation (Daly & Hazzledine, 1962), would not have been evoked. On the other hand, venodilatation, which often accompanies arteriolar dilatation in physiological responses, may be evoked by stimulation of the carotid bodies, and if so the volume of the venous reservoir would be expected to increase with the result that the venous return and venous pressure would tend to be reduced, thereby off-setting the rise in cardiac output. While not ruling out such a contingency, our observations on the changes in 'central' blood volume would suggest that pooling of blood in the systemic circulation is not a prominent feature of the vascular response to carotid body stimulation. Another mechanism which may antagonize the increase in cardiac output is bradycardia, which was evident in a few experiments, and may be accompanied by a negative inotropic effect on the heart (Downing *et al.* 1962).

In contrast to the response in spontaneously breathing dogs, stimulation of the carotid bodies invariably caused a reduction in cardiac output when the frequency and depth of breathing were maintained constant. This response is undoubtedly secondary to the primary reflex bradycardia which is revealed under these conditions, because it was reversed by procedures such as vagotomy or administration of atropine, which considerably reduced the slowing. It is suggested that the increase in cardiac output, which occurs in the artificially ventilated vagotomized preparation, is due largely to venoconstriction, since hexamethonium abolishes the response. In the artificially ventilated animals the reflex bradycardia and peripheral vasoconstriction occurring on stimulation of the carotid bodies would tend to evoke, therefore, diametrically opposite effects on cardiac output.

Mechanisms giving rise to secondary cardiovascular effects

Our experiments on spontaneously breathing animals provided information concerning some of the mechanisms by which hyperventilation, evoked reflexly by carotid body stimulation, causes secondary effects on the cardiovascular system which tended to mask the primary cardiac and vascular reflexes from the chemoreceptors. Two such mechanisms have so far been identified: the first is a reflex from the lungs, and the second is a reduction in arterial blood P_{CO_2}. When tests of chemoreceptor stimulation are made after denervation of the lungs or under conditions in which the arterial

blood P_{CO_2} is maintained constant, the primary reflex cardiac and vascular responses become more apparent. The results are even more striking when the arterial blood P_{CO_2} is maintained constant in preparations in which the lungs are denervated. Thus the cardiovascular responses to chemoreceptor stimulation in spontaneously breathing dogs, whose lungs are denervated and whose arterial blood P_{CO_2} is maintained constant, are usually identical with those elicited in dogs with controlled ventilation. It should be pointed out, however, that these two mechanisms cannot be the only ones involved, since in one of three experiments made on spontaneously breathing dogs the primary cardiac and vascular reflex effects of chemoreceptor stimulation were not revealed by combined denervation of the lungs and maintenance of a constant arterial blood P_{CO_2}. The additional mechanisms which might contribute to these secondary effects have yet to be identified.

If this interpretation of our results is correct, then it would be expected that inflation of the lungs *per se*, or a reduction in arterial blood P_{CO_2}, would cause cardiovascular effects similar to those observed on stimulation of the carotid bodies in spontaneously breathing dogs, namely, tachycardia, an increase in cardiac output and a decrease in peripheral vascular resistance. Evidence in support of this contention will now be discussed.

The reflex from the lungs. It is well established that an increase in inspiratory volume of the lungs causes tachycardia which is abolished by division of the pulmonary branches of the thoracic vagosympathetic nerves (Hering, 1871; Anrep *et al.* 1936*a*; Daly & Scott, 1958), but there is, as yet, little information as to the identity or location of the receptors giving rise to this response. More recently, Salisbury, Galletti, Lewin & Rieben (1959) have shown that in the dog inflation of the lungs causes reflex systemic vasodilatation, the afferent pathway lying in the cervical vagosympathetic nerves. It is evident, therefore, that our findings concerning the modification, by denervation of the lungs, of the cardiovascular response to chemoreceptor stimulation are supported by direct evidence concerning the reflex effects of lung inflation on the cardiovascular system.

Reduction in arterial blood P_{CO_2}. Induced hyperventilation in the cat, the dog and man causes a fall in blood pressure, increase in cardiac output and a decrease in total peripheral vascular resistance (Henderson, 1908; Dale & Evans, 1922; Burnum, Hickam & McIntosh, 1954; Richardson, Wasserman & Patterson, 1961). These responses have been attributed to a diminution in arterial blood P_{CO_2} because they can be reduced or abolished by administration of small concentrations of carbon dioxide during the period of hyperventilation.

Our findings fall into line with these observations in that the increase in cardiac output and reduction in total peripheral vascular resistance evoked

by stimulation of the carotid bodies were less evident if the arterial blood P_{CO_2} was maintained constant. Although we have no information at present to suggest the exact mechanism by which the reduction in arterial blood P_{CO_2} causes a diminution in total peripheral vascular resistance during chemoreceptor stimulation, there are three possibilities which are worthy of mention. First, it may result from a direct depressant effect on the vasomotor centre (Dale & Evans, 1922). Secondly, some mechanism other than a nervous one may be involved. In this connexion it has been shown that a decrease in arterial blood P_{CO_2} causes vasodilation in the nerve-blocked forearm in man (Clarke, 1952; Burnum *et al.* 1954; Roddie, Shepherd & Whelan, 1957). Lastly, the fall in arterial blood P_{CO_2} may be producing its effects by causing a reduction in the discharge of impulses from the aortic body chemoreceptors, on the assumption that the aortic and carotid chemoreceptors evoke similar responses. At normal levels of arterial blood P_{CO_2} there is a small continuous discharge of impulses from the chemoreceptors, and this ceases when the P_{CO_2} falls to about 30 mm Hg (see Heymans & Neil, 1958). In the present experiments the arterial blood P_{CO_2} was usually higher than normal, owing to depression of the respiratory centre by the anaesthetic, and there might be, therefore, an appreciable resting discharge from the aortic bodies in our experiments.

An interesting feature of our experiments is the masking of the primary cardiac and vascular reflex responses from the chemoreceptors by the secondary effects arising as a result of the concomitant hyperventilation. This phenomenon seems to us to be all the more striking in view of the potency of these primary reflex responses. Daly & Scott (1958), for instance, found that an average reduction in heart rate of about 40 % occurred when the chemoreceptors were stimulated in dogs artificially ventilated. With regard to the primary vascular reflex response, stimulation of the carotid body chemoreceptors caused increases up to 110 % in total peripheral vascular resistance in the artificially ventilated 'vasosensory controlled perfused living animal' preparation (Daly & Daly, 1959). Yet in the spontaneously breathing dog these primary reflex effects are masked by secondary mechanisms evoked by the concomitant reflex hyperventilation. Thus stimulation of the carotid bodies initiates not only primary cardiovascular reflex responses but also a chain of complex biological events involving secondary reflex and humoral mechanisms, the sum of whose potencies exceeds that of the primary reflexes.

SUMMARY

1. An investigation has been made of the effects of stimulation of the isolated perfused carotid body chemoreceptors on heart rate, blood pressure and cardiac output, measured by the dye-dilution method, in anaesthe-

tized dogs breathing room air. The chemoreceptors were stimulated by changing the perfusate from oxygenated to hypoxic blood.

2. In dogs with natural respiration stimulation of the carotid bodies caused an increase in respiratory minute volume and usually tachycardia, an increase in cardiac output and a reduction in total peripheral vascular resistance.

3. When changes in pulmonary ventilation during stimulation of the carotid bodies were prevented by applying artificial respiration, bradycardia, a diminution in cardiac output and an increase in peripheral vascular resistance invariably occurred. These results show that the cardiovascular effects occurring in the spontaneously breathing dog are due, not to direct or primary reflex effects from the chemoreceptors, but largely to secondary mechanisms arising from the concomitant reflex hyperventilation.

4. Two mechanisms giving rise to secondary effects on the cardiovascular system and evoked by reflex stimulation of respiration have so far been identified. These are an inflationary reflex from the lungs and a reduction in arterial blood P_{CO_2}.

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