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FACTORS AFFECTING MOVEMENT OF EXCITATORY SUBSTANCES FROM PULMONARY CAPILLARIES TO TYPE J RECEPTORS OF ANAESTHETIZED CATS

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SUMMARY

1. Using phenyl diguanide (PDG) as an excitatory substance, the role of certain factors that could influence the movement of such substances across the pulmonary capillaries to the J receptors was studied in cats anaesthetized with sodium pentobarbitone. This was aided by using a new method for estimating continuously *in vivo* the concentration (C) of PDG in the blood of the pulmonary artery.

2. Reduction of pulmonary blood flow by partial occlusion of the inferior vena cava enhanced the responses of the J receptors to PDG significantly in twelve out of thirteen trials. These effects, which occurred at a time when pulmonary capillary pressure (PCP) had fallen, could be related to the increase in the estimated mean C of PDG over the first 3 s or to the Ct (concentration × time) area to 50% of peak C. The responses bore no relation to peak C or rate of rise of C.

3. The responses of the receptors to PDG increased significantly after three out of eight injections of PDG while the PCP was raised by partial occlusion of the mitral orifice; reduced responses were recorded after two injections. These results, showing relatively much weaker stimulation by PDG in spite of the enhanced level of J receptor excitability produced by the raised PCP itself, suggest that movement of PDG out of the capillaries to the J receptors must be influenced primarily by forces governing diffusion, not filtration.

4. In addition to C of PDG there appear to be other factors that influence the responses of the receptors to PDG.

INTRODUCTION

The J receptors are stimulated under certain conditions that produce interstitial oedema in the lungs (Paintal, 1969; Roberts, Bhattacharya, Schultz, Coleridge & Coleridge, 1986). They are also stimulated by several excitatory substances. Some of these, such as 5-HT (Paintal, 1957), histamine and prostaglandins (see Coleridge & Coleridge, 1984) are produced endogenously, but the ones that have been most extensively used are phenyl diguanide (PDG) (Paintal, 1955, 1957) and capsaicin (see Coleridge & Coleridge, 1984). However apart from some knowledge about dose–response relationships in the case of PDG (Anand & Paintal, 1980) and nicotine (Kou, Frazier & Lee, 1989) little is known about the various factors that could

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influence the excitation of the J receptors by a variety of such excitatory substances. For example, one possible factor is raised pulmonary capillary pressure (PCP). In fact it is known that conditions which lead to raised PCP stimulate the J receptors (Paintal, 1969; Coleridge & Coleridge, 1977). Therefore under such conditions of increased excitability of the receptors it would be expected that their responses to excitatory substances such as PDG would be enhanced. Similarly, the reverse should be true under conditions of reduced PCP. However there is actually no information available at present regarding this issue. As a first step we therefore attempted to study the responses of the receptors to a fixed dose of PDG during raised PCP by raising left atrial pressure through partial occlusion of the mitral orifice and during lowered PCP by reducing blood flow to the lungs. In these initial experiments much to our surprise we found that certain responses of the receptors to PDG were the opposite of what had been expected, i.e. the responses to PDG were enhanced during reduced PCP and occasionally reduced during raised PCP. In order to find an explanation for these unexpected responses, reported in the present paper, we were led to devise a method for recording continuously the estimated concentration of PDG in the blood going to the capillaries. This method, which was communicated briefly to the Physiological Society recently (Paintal & Anand, 1991), has enabled us to study in a qualitative way the role of filtration and diffusion forces in influencing the excitatory effects of chemical substances.

METHODS

The experiments were carried out on cats anaesthetized with 35 mg kg^{-1} sodium pentobarbitone (Sagatal, May & Baker) given intraperitoneally. A catheter was inserted into the right saphenous vein such that its tip lay in the right atrium. The catheter was used for injecting PDG into the right atrium. A catheter was also inserted in the left atrium through the left auricle after opening the chest. This was used for recording left atrial pressure as well as for injecting PDG. A catheter was also inserted into the descending aorta through the right femoral artery for recording aortic blood pressure. A fourth catheter was inserted through a left lobar branch of the pulmonary artery for recording the pulmonary artery pressure. A thermistor was also inserted through the same branch such that its tip lay in the pulmonary artery. This was used for measuring the cardiac output using the thermodilution technique (Fegler, 1954; Korner & Hilder, 1974). It was also used for estimating the concentration of PDG according to the principle described below. The position of the tips of all the catheters were determined post mortem after killing the cat with an overdose of sodium pentobarbitone. All pressures were measured with suitable Statham pressure transducers (P 23 BB for left atrial and P 23 Gb for the other pressures). The intratracheal pressure was recorded using either a P 23 BB transducer or a PM 5 transducer. All the above pressures were recorded after closing (but not sealing) most of the chest wall except for openings, on both sides of the chest, which were thermally insulated from the atmosphere with dry cotton wool. Artificial respiration was maintained with a respiratory pump (Palmer 'Ideal'); its expiratory outlet was usually kept under about 4 cm of water. The end-tidal CO_2 (about 24–28 mmHg) was monitored in some experiments using a Beckman CO_2 analyser (Sensor Medics, Medical Gas Analyser LB-2).

In seven cats in addition to the left atrial catheter, a latex balloon was also inserted into the left atrium. This was used for raising left atrial pressure by distending it with about 1 ml of CO_2. In all cats a loop made of nylon catheter material was passed around the inferior vena cava (IVC) just rostral to the diaphragm. This was used for producing partial occlusion of the IVC for reducing blood flow to the heart.

Measurement of cardiac output

The cardiac output was measured using the standard thermodilution technique (Fegler, 1954; Korner & Hilder, 1974). Thermodilution curves were recorded following injection of 0·5 ml of 0·9% NaCl at room temperature (about 24 °C) and the area under the curve integrated electronically

using part of the circuit made by Mr Harold Ead of St Bartholomew's Hospital Medical College, London (see Bower & Ead, 1976). These circuits were similar to those of Korner & Hilder (1974). The values of cardiac output reported herein were similar to those obtained under similar experimental conditions in an earlier investigation using Fick's method (Anand & Paintal, 1980) i.e. about 78 ml kg^{-1} min^{-1}.

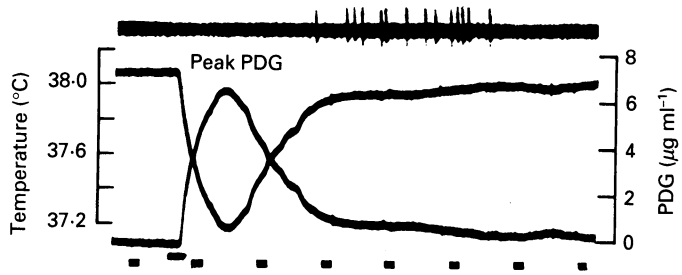

Fig. 1. Procedure for recording indirectly the rise rise in concentration of PDG in the blood of the pulmonary artery where the thermistor was placed. The downward curve shows the fall in temperature on injection of 0·5 ml NaCl solution containing 50 μg PDG into the right atrium at signal; delay between injection and arrival of injectate in the pulmonary artery was 0·2 s. This trace was inverted to show the rise in concentration of PDG (not the absolute concentration), its calibration on the right was obtained from eqn (1). The topmost trace shows the train of impulses in a J receptor produced by PDG. The lowest trace is of 1 s time marks and signal.

Estimation of changes in concentration of PDG in the pulmonary artery

The method used for estimating (albeit indirectly) the concentration of PDG in the pulmonary artery has been reported briefly (Paintal & Anand, 1991) and as already stated, the principle involved in this procedure is as follows: If n number of substances each weighing the same and *with identical physical properties* contained in the same injectate are injected into a blood vessel, then the concentration (C), at any particular time, of each one of the n substances at a point downstream from the injection site will be identical. Thus, the time–concentration curve for any one substance, for which a recording technique is available, will be representative of all the other substances. One of the 'substances' for which a recording technique is available is calories, i.e. temperature. Now, if a known quantity of a substance (e.g. PDG) is contained in the injectate and the thermodilution curve inverted as shown in Fig. 1, then its ordinate when appropriately scaled, will represent, according to the principle outlined above, the C of the drug in the time–drug concentration curve since the thermal indicator has, for the present purpose, the same physical properties as solutes, e.g. indicator dyes (Korner & Hilder, 1974). This is borne out by the fact that, as observed by Korner (1965) simultaneous measurements of cardiac output by the dye dilution and thermodilution methods yield identical or almost identical values of cardiac output.

The calibration scale for the concentration of the substance will be given by eqn (1) and the mean C over a certain period estimated by eqn (2).

$$C_b = T_b d_b s_b \frac{C_i}{d_i s_i (T_b - T_i) K} \tag{1}$$

and

$$\bar{C}_b = \frac{C_i}{d_i s_i (T_b - T_i) K} \times \frac{d_b s_b \int_{t_1}^{t_2} \Delta T_b(t)\, dt}{t_2 - t_1}, \tag{2}$$

where C_b is concentration of drug (μg ml^{-1}) in blood; $\bar{C}_b$, mean concentration of drug in blood (μg ml^{-1}); T, temperature; b, blood; i, injectate; d, density; s, specific heat; ΔT_b, change in temperature of blood (°C); $\int_{t_1}^{t_2} \Delta T_b(t)\, dt$, area between times t_1 and t_2; and K, correction factor relating to loss of thermal indicator in injection catheter (Korner & Hilder, 1974). Note volume of injectate which is part of the equation for measurement of cardiac output is not required in this

equation nor is it necessary to include the amount of drug in the injected volume; here the relevant element is the C of the drug expressed as milligrams or micrograms per millilitre. The C of PDG in the injectate at room temperature was 100 μg ml^{-1}; usually 50 μg contained in 0·5 ml of solution was injected. Both peak and mean C ($\bar{C}$) were measured from the PDG C curves (e.g. Figs 1 and 4). Using electronic integration and eqn (2), $\bar{C}$ was computed over the first 1 s after the arrival of PDG in the pulmonary artery (PDG $\bar{C}_{0-1s}$). Similarly $\bar{C}$ cover the first 2 s (PDG $\bar{C}_{0-2s}$) and first 3 s (PDG $\bar{C}_{0-3s}$) were computed. Finally the PDG $C \times$ time area (μg ml^{-1} s) to 50% peak C (see inset in Fig. 5) was measured (PDG Ct to 50% peak C). This provided a measure of the mean C over that period multiplied by the time.

Sources of error

As in the case of cardiac output measurements with the thermo-dilution technique the main source of error arose from the improper placement of the thermistor tip in the pulmonary artery, e.g. involving intimate contact with the wall of the pulmonary artery (occasionally, invagination into the wall). This was established post mortem. In such cases the measurements of cardiac output as well as the estimation of the C of PDG were discarded.

It should be noted that the PDG C provided by the present method indicates the actual level of C after the first injection only. In the case of subsequent injections the initial levels of PDG present in peripheral venous blood, as the residual amount remaining after previous injections, has to be determined if the actual amount of PDG (not just the change in PDG level as provided by the present method) needs to be known.

Pulmonary capillary pressure

The pulmonary capillary pressure was estimated from recorded values of pulmonary artery and left atrial pressures using a formula derived from a consideration of the relative values of resistances in the arterial and the venous sides of the pulmonary circulation observed by Gaar, Taylor, Owens & Guyton (1967). This formula, used by Snashall, Weidner & Staub (1977) is as follows:

$$\mathrm{PCP} = P_{\mathrm{la}} + 0{\cdot}4(P_{\mathrm{pa}} - P_{\mathrm{la}}),$$

where P_{la} and P_{pa} are the pressures recorded in the left atrium and pulmonary artery respectively and PCP is the estimated pulmonary capillary pressure.

Identification of J receptors and recording set-up

All the J receptors were identified using standard criteria, i.e. that they should not be stimulated by injections of about 100 μg PDG into the left atrium, that they should be stimulated by similar injections in the right atrium within 2·5 s, and that they should be stimulated smartly by insufflation of halothane vapours (Paintal, 1969). The arrangements for recording impulses from them in filaments dissected off the vagus nerve near the nodose ganglion and for identifying individual impulses using a special display system were similar to those used earlier (Anand & Paintal, 1980). The pre-amplifiers used were either Isleworth type 102 or Tektronix type 122. The impulses, along with other selected physiological variables such as aortic pressure, pulmonary artery pressure, left atrial pressure, intratracheal pressure, blood temperature and 1 s time marks and time (using a Racal GRA 0II-type time generator), were first recorded on a Racal tape-recorder (DS7) and subsequently photographed using a Tektronix type 7704A oscilloscope and a camera with continuously moving 70 mm photographic paper. Simultaneously certain selected pressures were monitored on a Beckman type RS dynograph.

Analysis of results

Measurements relating to latency, maximum intensity of discharge (i.e. maximum number of impulses in 1 s), duration of discharge, and the total number of impulses generated were made directly from photographic records. Measurements of latencies for stimulation of J receptors after injecting PDG were made from the moment PDG C rose in the pulmonary artery to the generation of the first impulse of the train.

Drugs

White crystalline powder of L-phenyl diguanide HCl (Koch-Light Laboratories Ltd) was used in a concentration of 100 μg ml^{-1}.

Statistical analysis

The significance of the difference of a particular measurement (e.g. during reduced blood flow) was established at 5% or 1% level if it exceeded the upper limit of the control mean value by 2·0 × S.D. or 3·2 × S.D. respectively. The significance levels of the correlation coefficient r were read from Fisher's Table (Fisher, 1958).

Protocol

At the start, about two to four control responses of a J receptor to a fixed dose of PDG (usually 12–18 $\mu g\ kg^{-1}$) given at intervals of about 4 min were recorded. Blood flow to the lungs was then reduced, so as to reduce the PCP, by partially occluding the IV. About 30 s later the same dose of PDG was injected and the occlusion released after recording the response to PDG. After about 4–5 min another set of control responses to PDG (usually two or three) were recorded. The means and S.D. of the control responses (usually five to seven, but two to three in two experiments) were determined and set down in Figs 3 and 5. The same procedure was followed when the PCP was raised by partial occlusion of the mitral orifice (Fig. 2).

RESULTS

Effects of raising PCP on J receptor activity

The effect of raising the PCP by a mean value of 8·6 mmHg (standard error of the mean, S.E.M. 2·4 mmHg) from an initial level of 10·7 mmHg (S.E.M. 2·6 mmHg) to 19·3 mmHg was examined on six J receptors during eight partial occlusions of the mitral valve. All the receptors were silent to begin with. As expected from the observations of Coleridge & Coleridge (1977) this was followed by the onset of naturally produced activity consisting of about 1 impulse s^{-1} in two receptors; in a third receptor occasional impulses appeared. In the remaining three receptors involving five occlusions no observable activity was produced by the raised pressure itself although subthreshold increase in excitability is to be expected.

Responses to PDG during raised PCP

Under the above conditions of increased excitability, the total number of impulses produced by the fixed doses of PDG increased significantly ($P < 0·05$) over the control level in only three out of eight trials (38%) as indicated by the data in Fig. 2*B*). It is noteworthy that in two receptors the responses were less than the control ones (two points below the line of identity in Fig. 2*B*). The increases in the maximum intensity of discharge produced by PDG during the occlusions (Fig. 2*A*) were similar ($P < 0·05$ in three receptors); it was less than control in two receptors.

Effects of reducing PCP on J receptor activity

Reduction of PCP was produced by partial occlusion of the IVC. This resulted in a mean fall of 4·6 mmHg (S.E.M. 0·37; $n = 13$) from an initial level of 11·5 mmHg (S.E.M. 0·65) before occlusion. The reduction in the PCP could be attributed to reduction in pulmonary blood flow. In this connection it should be recalled that the J receptors are stimulated when pulmonary blood flow is increased (Anand & Paintal, 1980). Accordingly, on-going activity in the receptors should fall on reducing blood flow but since there was no such activity in any of the nine receptors the expected effect of occluding the IVC was not seen during any of the thirteen occlusions. However, it is to be expected that the subthreshold level of excitability of these receptors should have fallen.

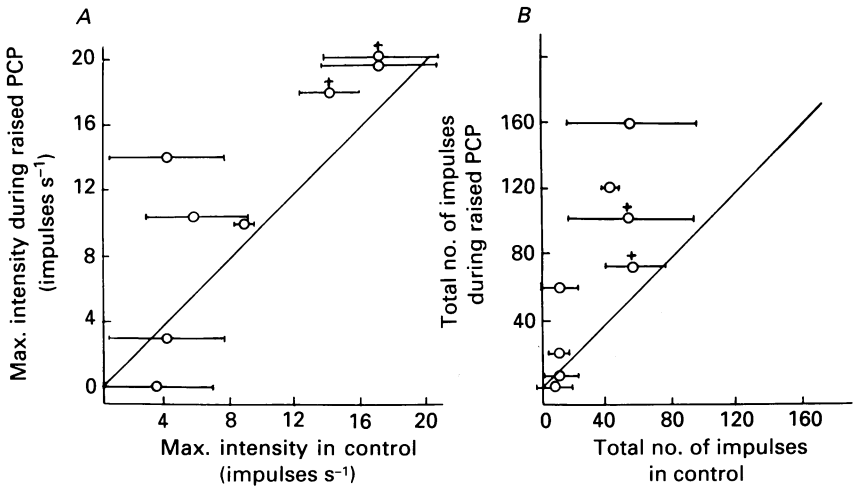

Fig. 2. Comparison of the mean responses of J receptors to a fixed dose of PDG under control conditions (abscissa, bars are ±S.D.) with those obtained while the PCP was raised (one injection only per occlusion of mitral valve, see text) using the same dose of PDG. + indicates receptors in which an on-going discharge was produced by raising PCP itself. *A*, maximum intensity of discharge; *B*, total number of impulses.

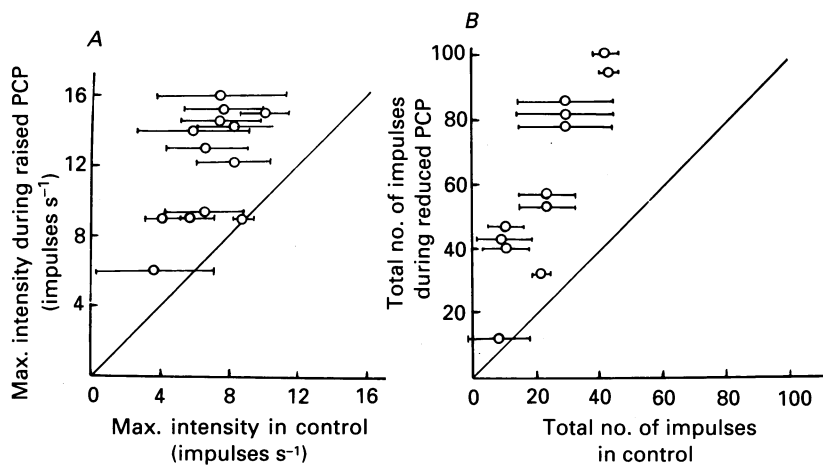

Fig. 3. Comparison of the mean responses of J receptors to PDG under control conditions (abscissa, bars are ±S.D.) with those obtained while the PCP was reduced (one injection only per occlusion of IVC) using the same dose of PDG. *A*, maximum intensity of discharge; *B*, total number of impulses.

Responses to PDG during reduced PCP

The responses of the receptors to a fixed dose of PDG were clearly enhanced (Fig. 3) when it was injected while the IVC was partially occluded. This occurred in the case of all the thirteen occlusions of the IVC involving nine J receptors; in no case was the response less than the control one. The enhanced responses were particularly significant ($P < 0{\cdot}01$) in 92% of the trials (12 out of 13) when the total number

TABLE 1. Effect of nine occlusions of the IVC on PCP, cardiac output, PDG C, and responses of six J receptors to PDG

Serial No. of occlusion	PCP		Cardiac output (% fall)	Level of significance of difference (P values) between control and occlusion values						
				PDG concentration				PDG Ct area to 50% peak C	Total No. of impulses	Maximum intensity
	Initial (mmHg)	Δ fall (mmHg)		Peak C P	$\bar{C}_{0-1s}$ P	$\bar{C}_{0-2s}$ P	$\bar{C}_{0-3s}$ P	P	P	P
1	14·8	6·4	44	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01
2	12·8	5·2	60	**	**	*	< 0·01	< 0·01	< 0·01	< 0·01
3	13·4	5·7	68	0·96	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	0·28
4	13·7	5·3	24	0·015	0·01	**	0·21	0·32	0·50	0·28
5	6·7	2·1	19	**	**	**	**	< 0·01	< 0·01	< 0·01
6	13·2	5·5	40	0·10	0·13	< 0·01	< 0·01	< 0·01	< 0·01	0·08
7	10·6	4·3	49	0·80	**	0·47	0·025	< 0·01	< 0·01	< 0·01
8	12·7	5·4	68	*	**	*	0·11	0·025	< 0·01	< 0·01
9	12·5	5·2	25	0·87	0·26	0·02	0·02	0·02	< 0·01	< 0·01

* Occlusion value same as control value, ** occlusion value less than control value.

of impulses produced by PDG during occlusion (ordinate) were compared with responses under control conditions (Fig. 3*B*). Although the maximum intensity of discharge during occlusion also increased, the increases were significant ($P < 0{\cdot}05$) in only eight out of thirteen occlusions (61 %) as shown in Fig. 3*A*. Nevertheless the

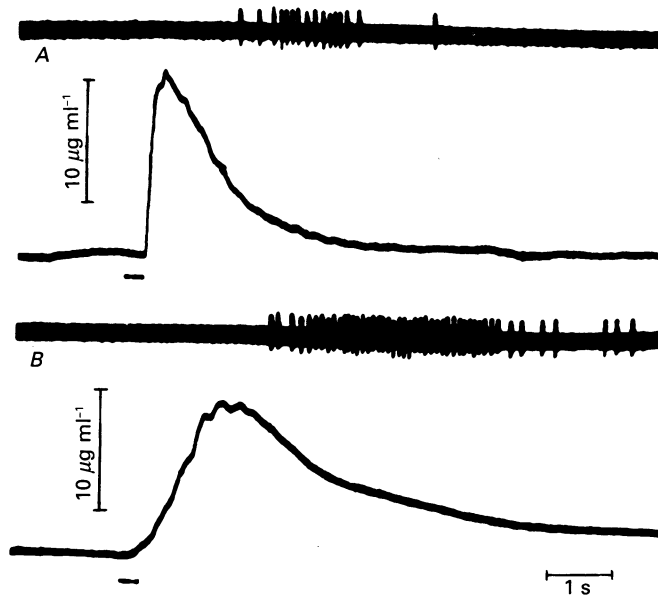

Fig. 4. Responses of a J receptor before (*A*) and while the PCP was reduced (*B*) to injection of 12 μg kg^{-1} PDG at signal into the right atrium; the second trace represents the estimated *C* of PDG (calibration bar on the right), note peak *C* was less in *B*. The mean estimated *C* over the first 3 s was 2·6 μg ml^{-1} in *A* and 3·5 μg ml^{-1} in *B*. Other data in Table 1 against serial No. 2.

increases in the responses to PDG during reduced PCP were far more impressive than during raised PCP (cf. Figs 2 and 3). This could be attributed to greater increases in the concentration of PDG while occluding the IVC than while occluding the mitral orifice (see Fig. 6 and related text in Discussion).

The above results suggest that neither increased excitability of the receptors arising out of raised PCP, nor increased filtration forces played an obvious positive role in the excitation of the J receptors by PDG because conditions leading to increased excitability of the receptors as indicated by the appearance of impulses after raising the PCP plus increased filtration forces (i.e. raised PCP) (Fig. 2) enhanced the responses to PDG clearly less than conditions which caused reduced excitability of the receptors as well as reduced filtration forces (Fig. 3). This, therefore, suggests that diffusion forces probably play the dominant role, i.e. the enhanced response of the receptors to PDG must depend on diffusion gradients. From this the questions that arise are the following: are the diffusion forces governed by the peak concentration (*C*) or by the mean *C* of PDG? In what way does the time factor influence the diffusion process?

Answers to the above questions could be sought in the case of nine injections of PDG during reduced blood flow (and their associated control records) in which records of cardiac output and estimated C of PDG were available. These have been included in Table 1 along with the significant increases in the total number of

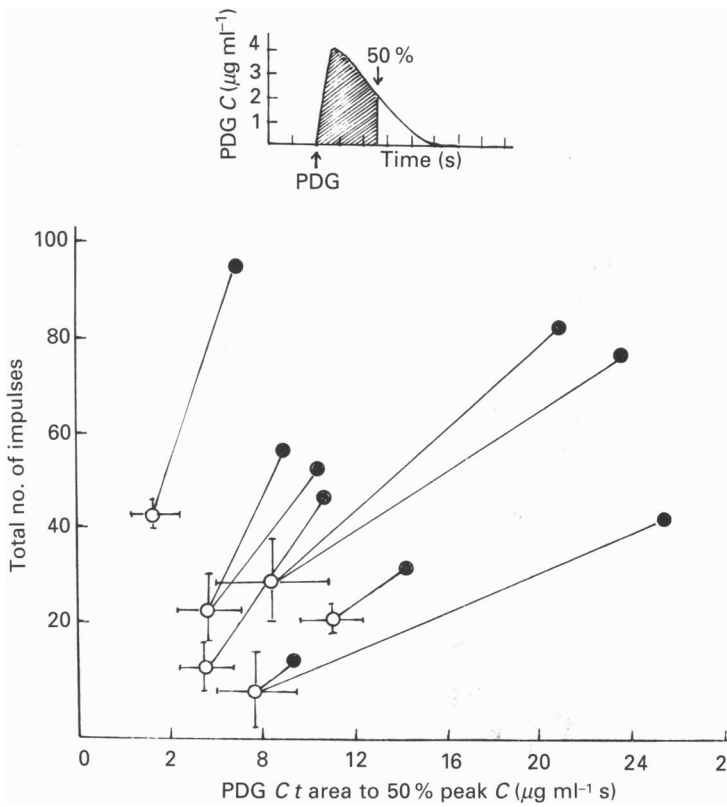

Fig. 5. Relation of total number of impulses (ordinate) produced by a fixed dose of PDG in six J receptors before (○) and during (●) nine occlusions of the IVC to PDG Ct area to 50% peak C (see inset), bars represent ±S.D. for the mean control values.

impulses produced by PDG during eight out of nine occlusions of the IVC. One example is provided in Fig. 4 which suggests that the enhanced response to PDG during reduced blood flow (Fig. 4*B*) cannot be attributed either to a higher peak C or to a greater rate of rise of C of PDG as the peak PDG C during reduced blood flow (Fig. 4*B*) was less than peak value under control conditions (Fig. 4*A*) (the mean control value was 6·6 μg ml^{-1}; $n = 6$). Similar observations were made during other occlusions. In only two out of the nine occlusions the peak C was significantly greater than control values (Table 1). In all the other trials where there was a small increase in peak C it was not significant (Table 1). This, therefore, cannot account for the significant increases in the total number of impulses produced by PDG during reduced blood flow.

Table 1 also shows that on the whole there was no significant difference between the control and occlusion values of mean C of PDG over the first 1 s, PDG $\bar{C}_{0-1s}$ or over the first 2 s (PDG $\bar{C}_{0-2s}$). However when mean C was evaluated over the first 3 s ($\bar{C}_{0-3s}$) then significant differences between control and occlusion values were noted in six out of the nine trials.

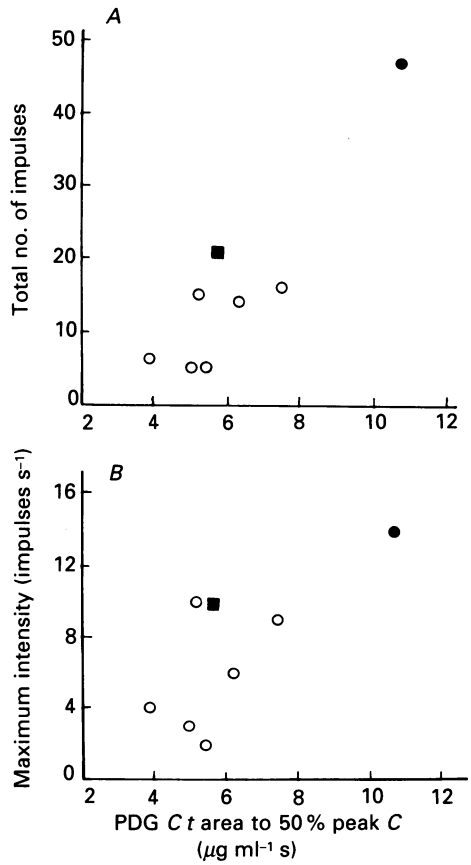

Fig. 6. Relation of responses of a J receptor to control injections (○) and to one injection of PDG during occlusion of the IVC (●) (reduced PCP) to the corresponding level of PDG Ct area to 50% peak C (abscissa). The squares in A and B relate to the response obtained while the PCP was raised; note the much lower Ct area than in the case of occlusion of the IVC.

These increased values were associated with the six significant increases in the total number of impulses produced by PDG during occlusion of the IVC (Table 1). In the analysis done so far, significant increases were observed most often in the case of values pertaining to the Ct area to 50% peak C which actually represents the mean C over this segment (not significant by itself) multiplied by the time to that point (Fig. 5). This suggests that both factors, i.e. mean C of PDG as well as the duration for which that mean C persists, may play a role in the stimulation of the

receptors by PDG. The mean control value after twenty-seven injections of 50 μg PDG was 6·8 μg ml^{-1} s (S.E.M. 0·5). The corresponding mean values for peak C was 7·0 μg ml^{-1} (S.E.M. 0·5) and for $\bar{C}_{0-3s}$ it was 3·1 μg ml^{-1} (S.E.M. 0·20).

Relation of responses to concentration of PDG under control conditions

It has already been indicated above (Table 1) that the large increases in the responses of the receptors during reduced blood flow were associated with large

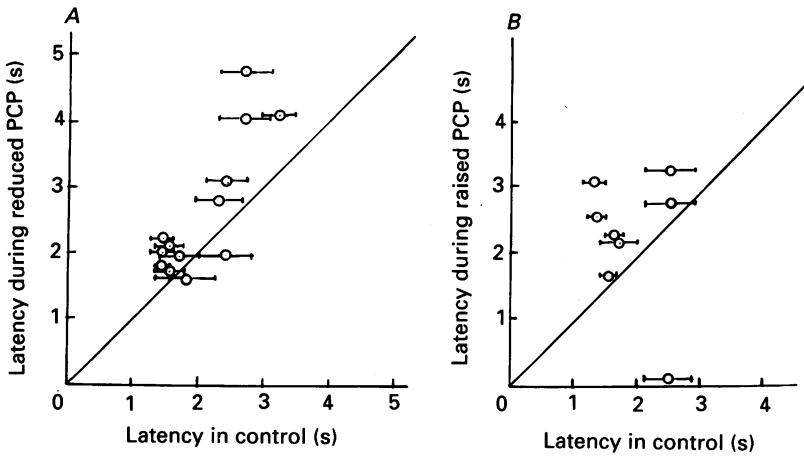

Fig. 7. Comparison of the mean latency for stimulation of J receptors under control conditions (abscissa, bars are ±S.D.) with those obtained while the PCP was reduced (*A*) of increased (*B*).

increases in PDG $\bar{C}_{0-3s}$ values and with increases in the Ct area to 50% peak C. However the variations *within* the control responses themselves do not provide firm evidence of being related to measurements of C. An example of the variations under control conditions in one receptor is shown by the points on the left-hand side of Fig. 6. On the face of it these cannot be convincingly related to the Ct area (although a trend can be seen) as the correlation coefficient r was not significant even at the 10% level in any of the six receptors as the degrees of freedom were too small; more data are needed.

Latency of the responses to PDG

The variations in the latency of the responses of individual receptors to stimulation by PDG under control conditions are indicated by the S.D. bars (abscissa) in Fig. 7. From these data it is clear that under both conditions, i.e. a rise in PCP (Fig. 7*B*) there were significant increases, but no significant decreases, in the latencies ($P >$ 0·05). This occurred in four out of eight receptors when PDG was injected while PCP was raised (Fig. 7*B*) and in eight out of fourteen occlusions of the IVC when the PCP was lowered (Fig. 7*A*). In both cases whenever values of cardiac output could be obtained this increase in latency was associated with reduction in blood flow ranging from 7 to 68% below control level. The reduced speed with which the blood transports the PDG to the capillaries near the receptors therefore plays a part in increasing the latency for stimulating the receptors.

DISCUSSION

In the future it should become possible to record directly the actual concentration of PDG in the blood by, say, a 'PDG electrode'. However, in the absence of such direct measurements the indirect method used in the present investigation for recording continuously the estimated concentration of PDG has proved satisfactory and should on theoretical grounds represent the actual PDG concentration in the blood assuming of course that the mixing of PDG (or other solutes) with the blood is identical to thermal mixing of the blood with the injectate containing PDG. This is practically certain (see Korner & Hilder, 1974). In addition the method has eliminated uncertainties and errors arising from measurements of latencies using injection signals. Thus it has helped to reveal the role of reduced blood flow in increasing the latency for stimulation of the J receptors by PDG (Fig. 7).

Role of diffusion and filtration forces

The present results have provided evidence to show that a rise in pulmonary capillary pressure *per se* apparently does not enhance the responses of the receptors to PDG since conditions causing the reverse enhance the responses to PDG more (cf. Figs 2 and 3). However, an unequivocal conclusion can be drawn if the responses of the receptors at raised PCP are compared with those at reduced PCP while keeping the mean concentration of PDG the same in both cases. This could not be done systematically with the present experimental arrangement. However, by chance, we obtained pairs of observations in two cats with control and raised PCP but at about the same mean level of PDG concentration. Data from one of them are shown in Fig. 6 which indicate that a raised PCP did not obviously enhance the response of the receptors even when the level of mean concentration of PDG was a little higher than the mean control level. In a second receptor the lack of effect of a raised PCP was even more impressive as the receptor was not stimulated at all after two injections of PDG during raised PCP even though the Ct area (8·5 μg ml^{-1} s) was greater than that during control injections (7·7 μg ml^{-1} s) which yielded an average of 5·5 impulses (range 0–17). Altogether the results suggest that filtration forces do not seem to play an obvious role in transporting PDG to the receptor site. It follows that diffusion forces must be the principal ones concerned with the transport of PDG (or similar substances) from the pulmonary capillaries to the J receptors.

Reasons for variations in responses to PDG under control conditions

Noteworthy variations in the responses of the receptors under control conditions have been observed as indicated by the S.D. bars in Figs 2, 3 and 5. However, it has not been possible to establish that the variations in the responses under control conditions can be accounted for by the corresponding variations in the concentrations of PDG. For example one of the better relationships between responses and PDG concentrations is shown in Fig. 6 and even from this it appears that the responses are poorly related to PDG concentration. Such results suggest that apart from PDG concentration there must be hitherto unknown factors that influence the excitation of the receptors by PDG or other excitatory substances. The phenomenon of tachyphylaxis does not seem to be one of them in the case of PDG at these low doses.

One wonders if variations in permeability of the capillaries could occur possibly under the influence of unknown mediators released from time to time under the existing experimental conditions; this would influence the excitatory effects of substances. One has also to consider factors that could influence the combination of the ligand with the 'receptors' on the sensory ending membrane on the events that follow. Clearly further studies are needed to explore the role of these unknown factors. Such studies may not only provide clues to the normal functioning of the receptors but may also help in understanding their role in the responses of the receptors to certain endogenously produced mediators under pathophysiological conditions. One factor seems to be time in the case of PDG as indicated by the data in Table 1 and Fig. 5. Finally as shown by the present results there was no evidence of additional enhancement of the responses to PDG when the subthreshold excitability of the receptors was increased even to the extent of generating 1 impulse s^{-1} (which is noteworthy activity in these receptors (Anand & Paintal, 1980) by the raised pulmonary capillary pressure itself. In this respect PDG behaves quite differently from histamine which reveals the presence of subthreshold activity in these receptors, presumably by acting on the voltage-gated channels, which manifests itself as sensitization of the sensory receptors to mechanical stimuli (Paintal & Anand, 1984).

The present results, e.g. the effects of reduced blood flow in enhancing the responses of the J receptors to PDG, indicate that it may be necessary to re-evaluate earlier results obtained during certain interventions as the effect of the intervention could have been secondary to changes in blood flow. For example in retrospect it could be that the enhanced effects of PDG seen during hypoxia (but not hypercapnia) (Anand & Paintal, 1980) could have been the result of increased concentration of PDG during hypoxia owing to possible reduction in blood flow and not to hypoxia *per se*. The same may apply to several other studies on sensory receptors and reflex effects involving excitation by chemical substances.

REFERENCES

ANAND, A. & PAINTAL, A. S. (1980). Reflex effects following selective stimulation of J receptors in the cat. *Journal of Physiology* **299**, 553–572.

BOWER, E. A. & EAD, H. W. (1976). An extrapolating analogue computer for measurement of cardiac output by thermodilution. *Journal of Physiology* **263**, 108–110*P*.

COLERIDGE, H. M. & COLERIDGE, J. C. G. (1977). Afferent vagal C-fibres in the dog lung: their discharge during spontaneous breathing, and their stimulation by alloxan and pulmonary congestion. In *Respiratory Adaptations, Capillary Exchange and Reflex Mechanisms*, ed. PAINTAL, A. S. & GILL-KUMAR, P., pp. 396–405. Vallabhbhai Patel Chest Institute, Delhi.

COLERIDGE, J. C. G. & COLERIDGE, H. M.(1984). Afferent vagal C fibre innervation of the lungs and airways and its functional significance. *Reviews in Physiology, Biochemistry and Pharmacology* **99**, 1–110.

FEGLER, G. (1954). Measurement of cardiac output in anaesthetized animals by a thermo-dilution method. *Quarterly Journal of Experimental Physiology* **39**, 153–164.

FISHER, R. A. (1958). *Statistical Methods for Research Workers*, thirteenth edn, revised, p. 209. Oliver and Boyd, Edinburgh.

GAAR, K. A. JR, TAYLOR, A. E., OWENS, L. J. & GUYTON, A. C. (1967). Pulmonary capillary pressure and filtration coefficient in the isolated perfused lung. *American Journal of Physiology* **213**, 910–914.

KORNER, P. I. (1965). The effect of section of the carotid sinus and aortic nerves on the cardiac output of the rabbit. *Journal of Physiology* **180**, 266–278.

KORNER, P. I. & HILDER, R. G. (1974). Measurement of cardiac output and regional blood flow by thermodilution. *Clinical and Experimental Pharmacology*, suppl. 1, 47–66.

KOU, Y. R., FRAZIER, D. T. & LEE, L.-Y. (1989). The stimulatory effect of nicotine on vagal pulmonary C-fibres in dogs. *Respiration Physiology* **76**, 347–356.

PAINTAL, A. S. (1955). Impulses in vagal afferent fibres from specific pulmonary deflation receptors. The response of these receptors to phenyl diguanide, potato starch, 5-hydroxytryptamine and nicotine, and their role in respiratory and cardiovascular reflexes. *Quarterly Journal of Experimental Physiology* **40**, 89–111.

PAINTAL, A. S. (1957). The location and excitation of pulmonary deflection receptors by chemical substances. *Quarterly Journal of Experimental Physiology* **42**, 56–71.

PAINTAL, A. S. (1969). Mechanism of stimulation of type J pulmonary receptors. *Journal of Physiology* **203**, 511–532.

PAINTAL, A. S. & ANAND, A. (1984). The detection of subthreshold activity of J and other receptors. In *Sensory Receptor Mechanisms*, ed. HAMANN, W. & IGGO, A., pp. 207–218. World Scientific Publishing Co. Pvt. Ltd, Singapore.

PAINTAL, A. S. & ANAND, A. (1991). Estimating *in vivo* the blood concentration of chemical substances injected intravenously in anaesthetized cats – use in studies on sensory receptors. *Journal of Physiology* **438**, 247*P*.

ROBERTS, A. M., BHATTACHARYA, J., SCHULTZ, H. D., COLERIDGE, H. M. & COLERIDGE, J. C. G. (1986). Stimulation of pulmonary vagal afferent C-fibres by lung edema in dogs. *Circulation Research* **58**, 512–522.

SNASHALL, P. D., WEIDNER, W. J. & STAUB, N. C. (1977). Extravascular lung water after extracellular fluid volume expansion in dogs. *Journal of Applied Physiology* **42**, 624–629.