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REFLEX EFFECTS FOLLOWING SELECTIVE STIMULATION OF J RECEPTORS IN THE CAT

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SUMMARY

1. Experiments carried out on anaesthetized cats showed that increasing blood flow, through the lobes of a lung, by 133 % (S.E. 33 %) generated an average of 0·75 impulses/sec (S.E. 0·3) in ten almost silent J receptors. Equivalent activity was produced by injecting 12–18 μg phenyl diguanide/kg into the right atrium. Such activity caused marked reflex effects, i.e. apnoea, rapid shallow breathing and reduction in the knee jerk.

2. The reflex effects of J receptors were studied after blocking the activity from cardiac receptors by intrapericardial injections of xylocaine. This was necessary because left atrial injections of phenyl diguanide produced reflex respiratory effects and inhibition of the knee jerk.

3. Hypoxia, but not hypercapnia, attenuated the reflex effects of J receptors, apnoea being abolished if the P_{a,O_2} fell below 35 mmHg. This was a central effect as it occurred in spite of increased activity of J receptors following phenyl diguanide, and effects of hypoxia persisted after cutting both carotid nerves.

4. The only invariable reflex effect of J receptors was a reduction in the total number and the average frequency of phrenic impulses in each breath. The changes in inspiratory time (t_i) and expiratory time (t_e) following apnoea were variable although most frequently both were reduced. In about half the observations the first effect before the apnoea was a reduction in t_i, in the other half it was a reduction in t_e. It was concluded that an input from J receptors inhibits inspiratory and expiratory mechanisms directly.

5. In some cats apnoea and rapid shallow breathing produced by J receptors continued after interrupting their activity by vagotomy and this did not diminish the reduction in t_i or t_e; in other cats it did. The reduction in t_e was at times quite independent of changes in t_i, i.e. pulmonary stretch receptor activity.

6. It was concluded that J receptors must be stimulated during moderate exercise to levels that produce marked respiratory reflex effects and inhibition of muscles.

INTRODUCTION

It would be desirable to study the reflex effects of type J receptors selectively by application of their natural stimulus which is, broadly speaking, pulmonary congestion (Paintal, 1969). Unfortunately, this has so far not been possible because pulmonary congestion also stimulates, to some extent, pulmonary stretch receptors (Marshall &

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Widdicombe, 1958; Costantin, 1959) and the rapidly adapting receptors, i.e. irritant receptors (Sellick & Widdicombe, 1969). Moreover, it is not easy to produce congestion in a consistently graded manner. Consequently, so far, the observations on the reflex effects of J receptors of the cat have been made largely by stimulating them with phenyl diguanide (Paintal, 1955; Deshpande & Devanandan, 1970; Schiemann & Schomburg, 1972; Schmidt & Wellhöner, 1970; Kalia, 1973; Winning & Widdicombe, 1976; Miserocchi, Trippenbach, Mazarelli, Jasper & Hazucha (1978). However, as has been pointed out, in these studies the doses used, typically, 150–200 μg have been excessive, as such doses produce activity of relatively high intensity, a level of activity that is generated in J receptors by pulmonary oedema (Paintal, 1973*a*). Consequently it was considered necessary to study the reflex effects using doses that would generate physiological levels of activity. Here a difficulty arose as it is not known what constitutes the normal range of physiological activity in J receptors, e.g. that produced by various levels of exercise which is considered to be a physiological trigger for stimulating these receptors by producing pulmonary congestion (Paintal, 1969, 1970). Therefore we first determined the level of activity produced in J receptors by increasing blood flow through the lungs as would occur by increasing cardiac output during exercise. This level of activity was then compared with that generated by various doses of phenyl diguanide and the reflex effects studied with this information at hand.

During the course of the study it was found that significant respiratory and somatic reflex effects could be produced in some cats by injecting phenyl diguanide into the left atrium presumably through stimulation of receptors in the heart. We therefore studied the reflex effects of J receptors after blocking the impulses from cardiac receptors with a local anaesthetic injected intrapericardially. Thus, through the above steps it became possible to study the reflex effects of J receptors selectively at physiological levels of activity of which reliable estimates were possible. The results have revealed that pronounced respiratory and somatic reflex effects are produced by physiological levels of activity in J receptors.

METHODS

The main results of this paper are based on observations in cats anaesthetized with sodium pentobarbitone (35 mg/kg) injected intraperitoneally. Some earlier observations on cats anaesthetized with chloralose 75 mg/kg (I.V.) have also been included; these will be mentioned specifically in the text. The experiments consisted of two series. In both of them a catheter was inserted through the right external jugular vein so that its tip lay in the right atrium. This was used for injecting phenyl diguanide.

Series 1 experiments. In these experiments on fifteen cats, impulses from individual fibres of type J receptors of the right lung were recorded using silver-silver chloride electrodes and a Tektronix 122 preamplifier which led to an oscilloscope (Tektronix 7704 A) and also to a six-channel tape recorder (Bell & Howell – VR 3200). Impulses were photographed on 70 mm Kodak recording paper during the experiment itself and also occasionally after the experiment from the tape recorder. The other records consisted of either intratracheal pressure (in the open chest experiments) or intrathoracic pressure recorded through a Malecot catheter or a wide-bore needle. The aortic pressure was recorded through a catheter inserted through the left common carotid artery so that its tip lay at the semilunar valves; this catheter was used for injecting phenyl diguanide into the aorta. The transducers used were Statham type 23 Gb. The positions of all catheters were confirmed post mortem.

The right side of the chest was first opened, while the cat was ventilated with a Palmer respiratory pump, the stroke volume having been adjusted to keep the end tidal P_{CO_2} at about 25–30 mmHg. The right vagus was cut below the lungs and a catheter inserted into the pulmonary artery through a branch of the artery in the diaphragmatic lobe of the right lung. This lobe (as well as the azygos lobe) was then tied off so that no blood or air entered or left the lobe. The chest was then closed. Throughout these procedures special care was taken to ensure that the remaining lobes, i.e. apical and middle, of the right lung were not handled.

After closing the right side of the chest, the left side was opened through the fourth or fifth intercostal space and the left pulmonary artery exposed so that it could be occluded with a bulldog clip when required for increasing blood flow through the apical and middle lobes of the right lung while recording impulses from J receptors located therein.

Estimation of blood flow through the lungs. The following procedure was adopted in order to estimate the blood flow through the first and second lobes of the right lung: the cardiac output was measured using the Fick principle. The expired air was collected for 30 sec from the respiratory pump and its volume measured. Its O_2 cocenteration was determined using a Scholander 0·5 ml. gas analysis apparatus for calculation of $\dot{V}_{O_2}$. Blood samples were taken from the right atrium and the aorta. The oxygen concentration in the blood was determined using the method of Linden, Ledsome & Norman (1965). Blood gas tensions were measured using a Radiometer PHM 73 pH/blood gas monitor. It was assumed that the entire cardiac output flowed equally through the lobes of the lungs whose pulmonary arteries were open, i.e. through both lobes of the left lung and the apical and middle lobes of the right lung with the left pulmonary artery unoccluded and only through the right lung lobes when the left pulmonary artery was occluded. At the end of the experiment the left lung lobes and right-sided lobes were cut at the hilum and weighed separately immediately after cardiac standstill had been produced by injecting excess of sodium pentobarbitone and after wiping off any blood with filter paper. Blood flow through the lungs was then estimated by dividing the cardiac output by the weight of the lung lobes through which blood flowed and expressed as ml. blood/min/per g of lung tissue. The measurements always consisted of a pair, i.e. one estimate of blood flow with the left pulmonary artery open and the second with the left pulmonary artery closed (blood diverted to the right lung lobes).

Identification of J receptors. Fibres were assumed to belong to J receptors if a discharge appeared within 2·5 sec of injection of phenyl diguanide into the right atrium and no activity was generated when it was injected into the aorta or left atrium. In most instances further confirmation was obtained by noting that it was stimulated within 0·3 sec following insufflation of halothane into the trachea (Paintal, 1969).

Series 2 experiments. This series consisted of twenty-five cats in which the reflex effects of injecting phenyl diguanide were examined. In addition to the other records mentioned under Series 1 experiments, the knee jerk was also recorded as described by Kalia (1973). Another addition was the use of an ink recorder (Beckman-Dynograph) along with photographic recording and records on tape (see Series 1 experiments) for recording the intrathoracic pressure (through a Malecot catheter) and blood pressure or the knee jerk. The catheter used for recording the blood pressure was inserted through the femoral artery.

The left side of the chest was opened and a catheter was inserted into the left atrium through the auricular appendage after making a slit in the pericardium. In addition another catheter of 1 mm external diameter was inserted into the pericardial space through the same slit in the pericardium which was subsequently closed with a purse-string suture. Care was taken to ensure that no vessels in the pericardium were left open so as to prevent absorption of xylocaine through these vessels. This catheter was used exclusively for injecting 2 % xylocaine into the pericardial cavity. Finally the chest was closed and the cat breathed spontaneously.

In eight of the cats of this series efferent impulses from the central end of a slip of root V of the phrenic were also recorded on one channel of the oscilloscope and the tape recorder. In addition, the total number of phrenic impulses in each breath were counted using a Tektronix 7D 15 universal counter timer plug-in unit and the counts displayed on the oscilloscope and photographed. Simultaneously, the impulses were displayed as an average frequency of discharge using bin intervals of 50 or 100 msec (Figs. 6 and 9). The total count of impulses obtained through this record corresponded with that displayed on the read-out system through the 7D 15 counter-timer unit. If the correspondence was absent due to untimely triggering of the read-out circuit, the latter reading was discarded.

The device for recording the average frequency of impulses was stable and linear. The circuit

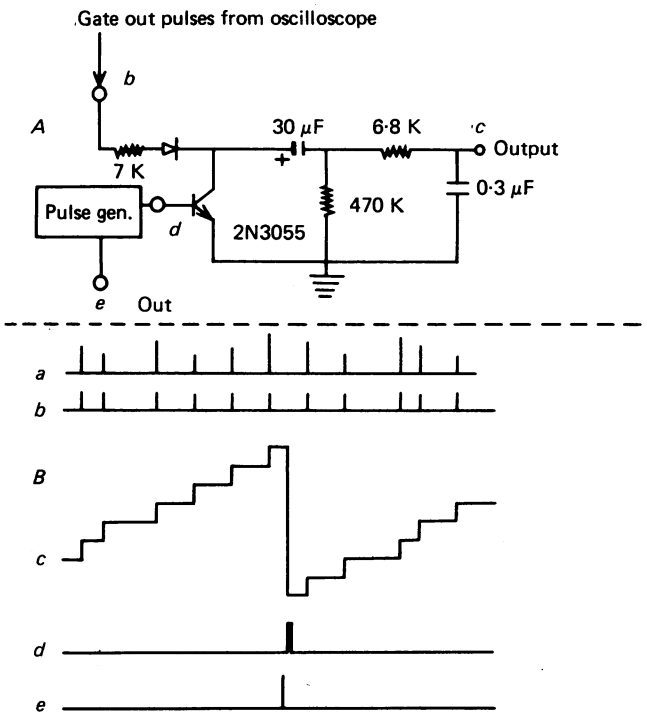

Fig. 1. Diagram of circuit (*A*) and wave forms generated by it (*B*) at various points of the circuit used for obtaining a record of the average frequency of discharge as shown in Figs. 6 and 9. For description and operation of the circuit see text.

of this device is shown in Fig. 1*A*. It was based on charging a 30 μF capacitor by pulses of fixed duration and amplitude produced by each of the phrenic impulses and discharging this capacitor at intervals of either 50 or 100 msec. The wave forms generated at different points in the circuit are shown in Fig. 1*B*. Each impulse *a* generated a gate pulse of 0·5 msec in the sweep circuit of the oscilloscope. These gate pulses *b* were used to charge the 30 μF capacitor in a stepwise manner shown in *c* so that each pulse produced an increase in voltage of the same amplitude at the output of the circuit on the receipt of each pulse. The voltage produced by any pulse could remain unaltered for over 100 msec because of the long time constant of the circuit (Fig. 1*A*). The charges produced by each pulse summated up to 49·9 or 99·9 msec *c* when a positive pulse *d* of about 1 msec duration applied to the base of the shorting transistor (2N 3055) drove it hard on and discharged the capacitor immediately. About 0·1 msec before the capacitor was discharged, a positive pulse *e* was applied to the Z axis to unblank one of the beams of the oscilloscope, thereby producing a spot on the oscilloscope. This spot whose height depended on the summed charges between 0 and 99·9 msec (or 49·9 msec at bin intervals of 50 msec) represented the average frequency of discharge as shown in Figs. 6 and 10. Pulses *d* and *e* were generated by a free-running pulse generator at a frequency of either 10 Hz (bin interval of 100 msec) or 20 Hz (bin interval 50 msec). From the records e.g. Figs 6 and 9) it was possible to determine the total number of efferent impulses during each inspiratory burst of the phrenic. This was more reliable than the counts of impulses indicated on the read-out system by the 7D 15 plug-in unit because of occasional untimely triggering of the read-out system during the respiratory cycle by the intrathoracic pressure signal. It should be noted that the total count depended on the size of the spikes. If they increased in size, e.g. owing to movement of the filament across the electrodes then the total count increased as apparently happened in the case of Fig. 9*B*. Therefore the counts obtained in one record were not compared in absolute terms with those obtained in another record. Instead the counts were expressed as percentage of control for purposes of comparison (e.g. Fig. 8).

TABLE 1. Effect of occluding the left pulmonary artery on the activity of J receptors (averaged per 10 sec), pulmonary artery pressure, cardiac output and blood flow in the right lung lobes

	Pulmonary artery pressure			Cardiac output		Blood flow in right lung lobes			Activity in J receptor		
Serial no. of cat	Before occlusion (mmHg)	30–60 sec after occlusion (mmHg)	Increase (%)	Before occlusion	30–60 sec after occlusion	Before occlusion (ml./min)	30–60 sec after occlusion (ml./min)	Increase (%)	Serial no. of receptor	Before occlusion (imp./sec)	30–60 sec after occlusion (imp./sec)
1	27	36	33	249	334	92	334	265	1	0·3	2·4
									2	0·0	0·8
2	23	44	91	207	246	75	246	227	3	0·3	1·0
2	—	—	—	215	132	79	132	68	4	0·0	0·0
									5	0·0	0·0
3	19	28	47	309	143	113	143	27	6	0·0	0·6
4	29	40	38	213	145	68	145	113	7	0·0	0·0
									8	0·0	1·1
4	25	43	72	372	226	123	226	83	9	0·0	0·0
5	29	42	45	242	204	83	204	147	10	0·0	3·0
Mean	25·3	38·8	54·3	253	204	90	204	133·9		0·06	0·9
S.E.	±1·6	±2·5	±9·2	±23·1	±27·4	±7·7	±27·4	±32·7		±0·04	±0·3

Solutions. Phenyl diguanide (Koch Light Laboratories) in concentrations of 100 μg/ml. kept at about 38 °C was used. Xylocaine 2 % (Astra Laboratories) was used for injections into the pericardial space.

Analyses. Measurements of inspiratory time (t_i) and expiratory time (t_e) were made from the average frequency of discharge (see Figs. 6 and 9) and intrathoracic pressure traces. The time between the sudden rise to the sudden fall in the average frequency trace was taken to be a measure of t_i. The time between the sudden fall to the start of the next cycle was taken to represent t_e. Alternatively measurements were made from the corresponding points of the intrathoracic pressure trace.

Index of respiratory inhibition. The duration of apnoea produced reflexly by phenyl diguanide could have been used as a simple index of respiratory inhibition but it proved unreliable owing to the fact that weak respiratory movements, as indicated by the bursts of activity in the phrenic, appeared during the period of so-called apnoea (see Figs. 6*B* and 9*A*). The duration of reduction of phrenic activity to 50 % of control was therefore used as an index of respiratory inhibition and this was assessed against the duration of the control respiratory cycle. This has been termed the inhibitory ratio, i.e.

$$\text{Inhibitory ratio} = \frac{\text{duration of 50 \% reduction of phrenic activity}}{\text{average duration of control respiratory cycles}}.$$

RESULTS

Series 1 experiments

Effect of increase in pulmonary blood flow on J receptors. The effect of increase in blood flow was examined on ten type J receptors (Table 1) in five cats according to procedures described in the Methods. Eight of these receptors were typically silent under control conditions; in two of them there was resting activity of about 0·3 impulses/sec (averaged over 10 sec) which is also to be expected (Paintal, 1969). Six of these receptors were stimulated on occluding the left pulmonary artery for about 1 min, four were not. The blood flow to the lobes of the right lung in which the receptors were located increased in every case, the percentage increase depending on the effect of occlusion on the cardiac output. The latter increased in two trials and fell in the remainder (Table 1). The increase in pulmonary blood flow which averaged 133 % produced variable increase in the activity of the receptors (Table 1); the correlation coefficient between the two was +0·47 but it was not significant ($0{\cdot}1 < P < 0{\cdot}2$) as shown by Student's t test.

In five out of six fibres the increase in activity set in within 10 sec and the activity increased gradually to reach a peak within 1 min. The degree of increase was variable as shown in Fig. 2. On releasing the occlusion the activity fell rapidly. In order to obtain an estimate of the average flow of impulses, the activity in all six receptors was integrated using the time of occlusion of the left pulmonary artery as the starting point. The summed impulses were divided by ten (total number of receptors examined) to get an estimate of the average activity. This represents the average rate of discharge in a population of receptors stimulated by phenyl diguanide. Fig. 3 shows that increasing blood flow by 133 % led to an average discharge of 0·75 impulses/sec between 40 and 60 sec after occlusion of the left pulmonary artery. This value is less than the mean maximum activity of 0·9 impulses/sec shown in Table 1 because the maximum activity appeared at different times as can be seen in Fig. 2.

Having found that increase in blood flow by 133 % produces average activity of the order of 0·75 impulses/sec in a population of receptors stimulated by phenyl

diguanide, the next step was to find out what dose of phenyl diguanide would lead to the same intensity of activity in a similar population of J receptors.

Relation of dose of phenyl diguanide to activity in J receptors. The effects of different

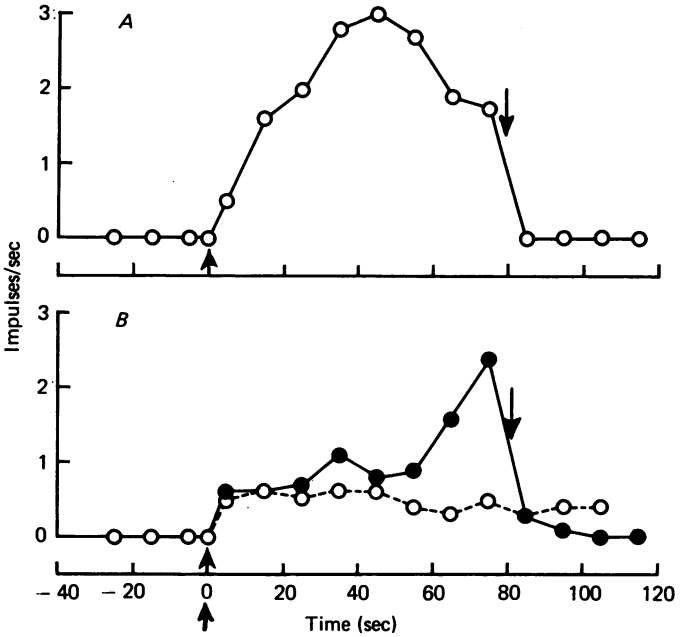

Fig. 2. Effect of increase in blood flow in right lung lobes on three J receptors of three cats by occluding the left pulmonary artery at upward arrow. In *A*, the blood flow increased by 147 % in *B* by 113 % in case of receptor shown by filled circles and by 27 % in the one shown by open circles. Downward arrows mark the release of the occlusion. The ordinate shows the frequency of discharge averaged over 10 sec. Note that maximum activity occurred at different times in the three receptors.

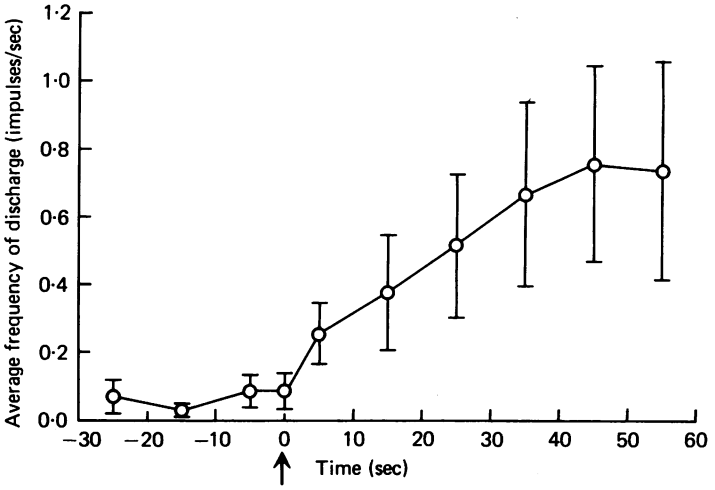

Fig. 3. Effect of increasing blood flow by an average of 133 % on the average activity of ten J receptors in the right lung. Blood flow was increased by occluding the left pulmonary artery at arrow. Vertical bars represent standard errors.

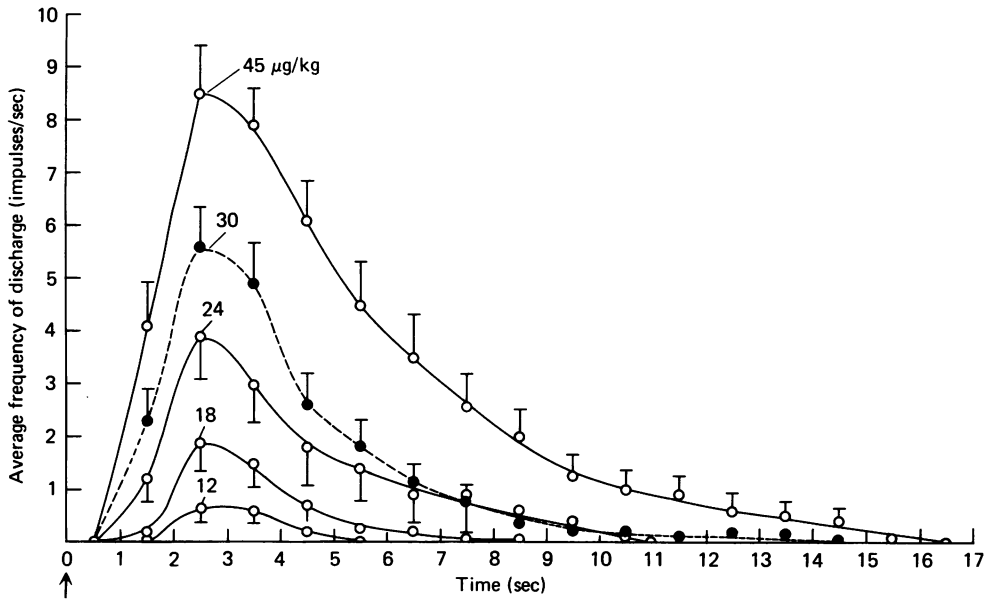

Fig. 4. Average activity in twenty-one J receptors produced by different doses of phenyl diguanide (indicated on the top of each curve) injected into the right atrium at arrow. Vertical bars represent S.E. The curve for 30 μg/kg has been shown differently so as to distinguish its points from those relating to the 24 μg/kg curve.

doses of phenyl diguanide on twenty-one J receptors in ten cats were recorded. The interval between injections was 3 min or more. Starting from 6 μg/kg the doses injected were increased in steps of 6 μg/kg to 30 μg/kg; the next dose after this was 45 μg/kg. The threshold dose was greater than 6 μg/kg in twenty receptors; one receptor yielded one impulse at this dose. The modal threshold dose was between 12 and 18 μg/kg. The impulses in all the twenty-one receptors produced by each dose was added and the mean discharge produced every second was determined. This is shown in Fig. 4, which indicates that peak frequency of discharge for all doses appeared about 2·5 sec after injection. This average peak frequency of discharge has been plotted against each dose in Fig. 5. Also shown in Fig. 5 is the average frequency of discharge for the main burst of impulses produced by each dose. This was obtained as follows: for each dose, all the impulses up to 90 % of the impulses produced by it were taken from the results of Fig. 4. The first impulse for each active fibre was deducted from this total, the average per fibre determined and this value divided by the time up to 90 % of the discharge. The last 10 % of the impulses generated by phenyl diguanide were excluded as, often, the last few impulses appeared as separate impulses at relatively prolonged intervals from the main burst. Inclusion of these impulses would have resulted in unduly reducing the average frequency of the discharge.

Series 2 experiments

Reflex effects of J receptors. The primary aim of these experiments was to study in isolation the reflex of J receptors at levels of activity that were equivalent to increas-

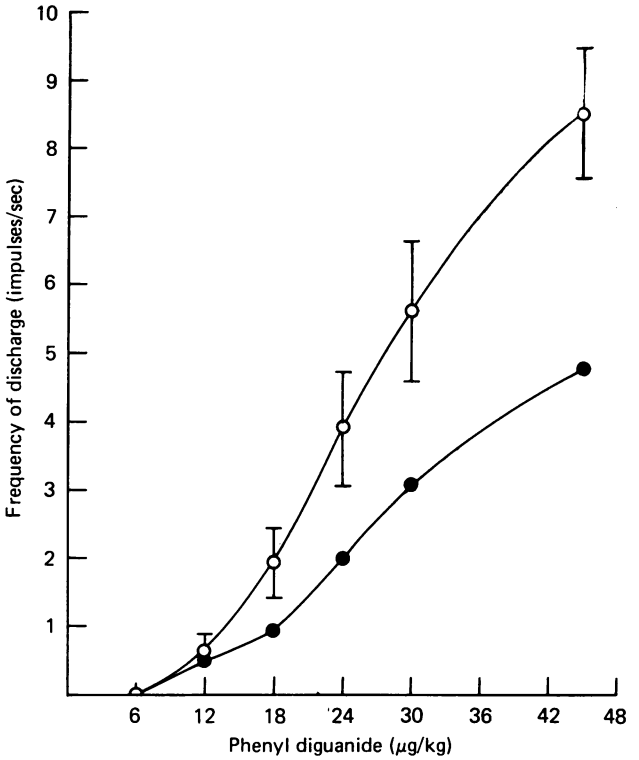

Fig. 5. Average peak frequency of discharge with S.E. (open circles) and average frequency of discharge in the bursts of impulses (filled circles) of twenty-one receptors produced by different doses of phenyl diguanide.

ing blood flow by 133 %, i.e. by injecting 12–18 μg phenyl diguanide/kg into the right atrium (compare Figs. 3 and 5). However, in every cat injection of such doses into the left atrium produced not only reflex bradycardia through stimulation of receptors in the heart (Dawes & Mott, 1950) but there also appeared reflex respiratory effects consisting of variable duration of apnoea or change in t_i or t_e and reduction of phrenic activity. Such reflex effects, from receptors in the heart, which were pronounced in some cats (e.g. Fig. 6*A*) were blocked by injecting 0·5–2·5 ml. 2 % xylocaine into the pericardial space (Fig. 6*B*). In several experiments total block was achieved but in some there remained some residual effects (e.g. Fig. 6*B*). The reflex effects of J receptors were then recorded not only at doses of 12–18 μg/kg but also at higher doses after blocking the cardiac receptors. An additional advantage of this procedure was that respiratory and somatic reflex effects of J receptors could be studied in the absence of the pronounced reflex bradycardia and hypotension produced by phenyl diguanide (Fig. 6 *D* and *E*). The injections of xylocaine were repeated when the block wore off.

Dose–reflex response relationship. In order to get an estimate of the relationship between different levels of sensory inputs from J receptors and the reflex respiratory effects produced by such inputs it was necessary to get a suitable criterion of reflex respiratory inhibition. As stated in Methods, the inhibitory ratio involving the

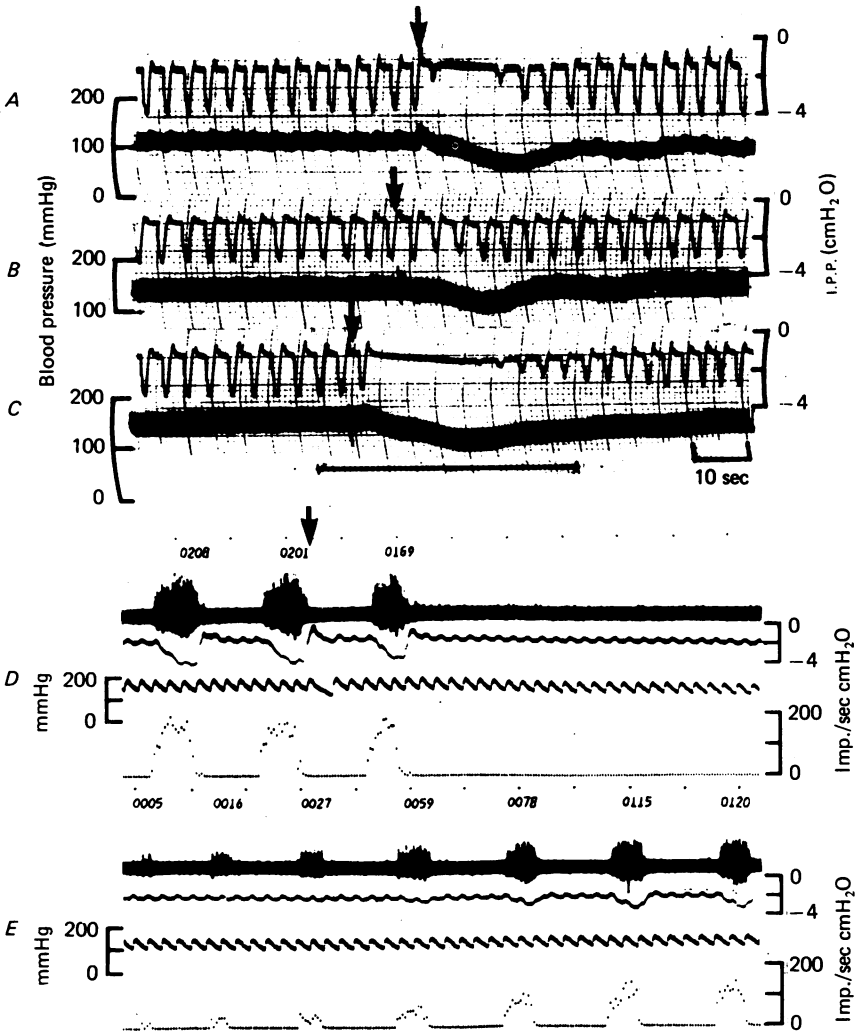

Fig. 6. *A* and *B* show the effect of injecting 30 μg/kg phenyl diguanide into the *left* atrium at arrows before and after blocking the impulses from cardiac receptors by injecting 2 % xylocaine into the pericardial space between *A* and *B*; note reflex apnoea seen in *A* is abolished in *B*. After the neural block the J receptors were stimulated by injecting 30 μg/kg phenyl diguanide into the *right* atrium at arrows and the reflex effects recorded simultaneously on the dynograph (*C*) and on photographic paper (*D* and *E*). Records *D* and *E* which are continuous were taken during the period marked by the straight line in (*C*). The traces in *D* and *E* are from above downwards, 2 sec time marks, count of phrenic impulses, intrathoracic pressure, aortic blood pressure and average frequency of impulses in a phrenic slip (bin duration 100 msec, calibration on the right). Note appearance of phrenic bursts during apnoea in *E*.

activity of the phrenic was used because the duration of apnoea proved to be an uncertain feature of the inhibition produced by phenyl diguanide. Moreover as reported earlier (Paintal, 1955) the smaller doses of phenyl diguanide did not produce apnoea but rapid shallow breathing only.

In Fig. 7 is shown the degree of inhibition of phrenic motoneurones produced by different doses of phenyl diguanide in two cats, one in a relatively lightly anaesthetized state (various reflexes brisk) and the other more deeply anaesthetized. In most experiments the dose–response relation shown for the lightly anaesthetized cat in Fig. 7 was seen, i.e. there was a levelling off of the reflex effects above 18 μg/kg. Also in most cats the threshold dose was about 12 μg/kg; in three cats weak reflex effects were visible at about 9 μg/kg. Fig. 7 also shows that although the sensory input produced by about 18 μg/kg (i.e. about 1 impulse/sec (see Fig. 5)) yields large reflex effects, it is the greater input of 1–5 impulses/sec (Fig. 5) from J receptors by the larger doses that is effective under certain conditions, e.g. deep anaesthesia.

Effect on mean impulse frequency of phrenic bursts. In all eight cats phenyl diguanide not only reduced the total number of phrenic impulses in each breath (e.g. Fig. 9) but it also reduced the mean frequency of discharge (total impulses in each breath divided by t_i) in all of them. For example in Fig. 6 *E* the mean frequency of discharge was 16–50 % of control in the first few breaths after apnoea. This contrasts with the observations of Winning & Widdicombe (1976) who made their observations on single fibres of phrenic.

Effect on t_i and t_e. In about three-quarters of the observations it was found that after the apnoea there was a reduction of both t_i and t_e in agreement with the observations of Winning & Widdicombe (1976) and Miserocchi *et al.* (1978). In the remainder there was a clear increase in t_e without an increase in t_i or associated with reduced t_i (e.g. Fig. 6*E*). In about half the observations, depending on the time of injection, it was found that the first effect before the apnoea, following injection of phenyl diguanide, was a reduction in t_i and a reduction in the amplitude of the breath following an unchanged preceding t_e. In the other half of the observations the reverse was seen, i.e. the first effect before apnoea was a reduction in t_e without any evidence

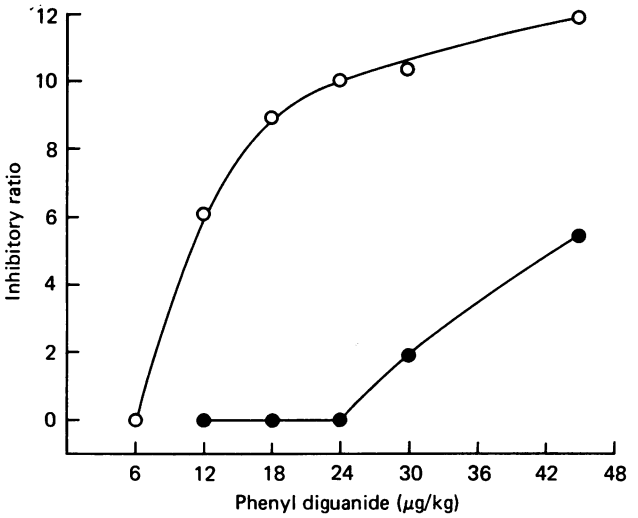

Fig. 7. Relation between dose of phenyl diguanide injected into the right atrium and the reflex effects produced by each dose. The inhibitory ratio on the ordinate represents the duration of 50% inhibition of phenric motoneurones divided by the duration of the contol of respiratory cycles. Open circles, lightly anaesthetized cat, filled circles, relatively deeply anaesthetized cat.

of preceding reduction in t_i or amplitude or the total phrenic activity of the preceding breath (e.g. Fig. 9*A*); reduction in both was always seen in the succeeding breath (e.g. seventy-nine phrenic impulses compared with 130 in Fig. 9*A*). Thus it is clear that reduction in t_e can occur independently of any change in t_i or the input of pulmonary stretch receptors.

Effect of vagotomy. In order to determine the role of the input from pulmonary stretch receptors on changes in t_i and t_e produced by J receptors the effect of vagotomy was examined in seven cats, six anaesthetized with chloralose, one with sodium pentobarbitone. To begin with, control observations with phenyl diguanide were

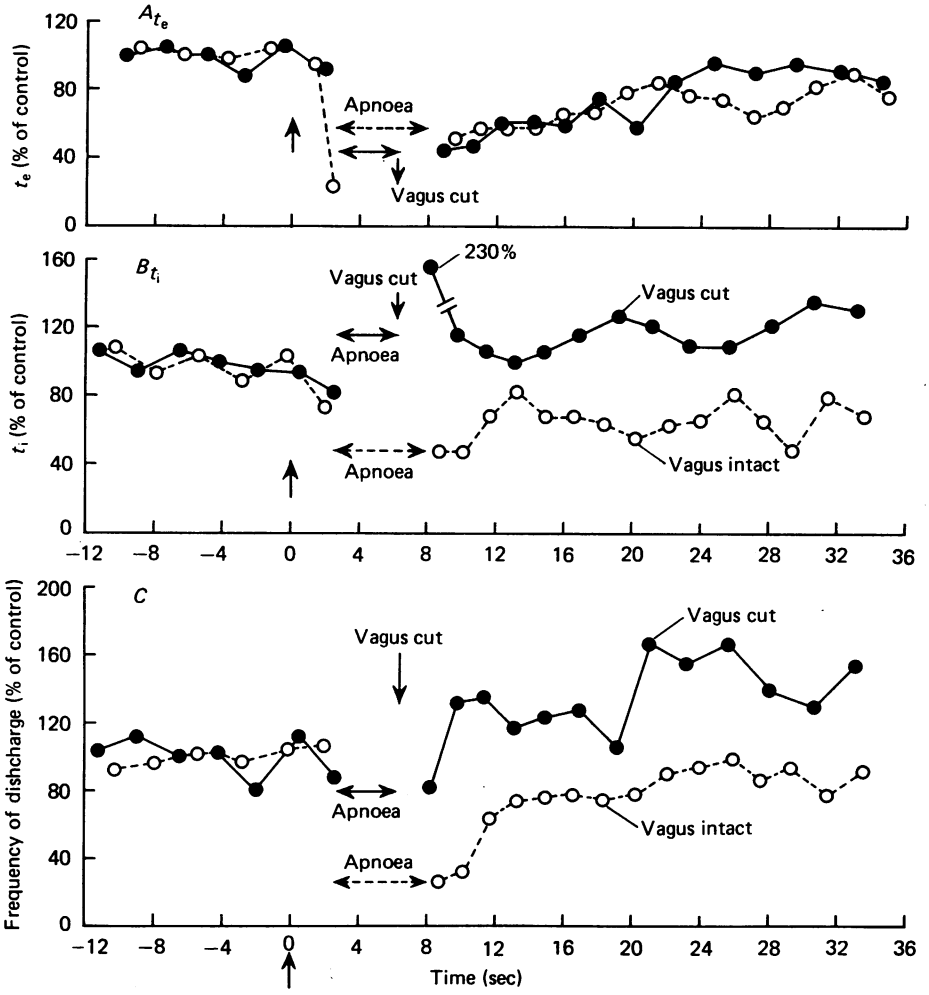

Fig. 8. Effect of cutting the right vagus (left already cut). The filled circles show the effect of cutting the right vagus 6 sec after injecting phenyl diguanide 30 μg/kg at arrow at zero time on t_e (*A*), t_i (*B*) and the mean frequency of discharge in phrenic motoneurones (*C*). The open circles show the effect of phenyl diguanide without vagotomy. The horizontal arrows indicate duration of apnoea with vagotomy (continuous line) and without vagotomy (interrupted line). The ordinates represent values expressed as percentage of control values before injection of phenyl diguanide into the right atrium.

made after cutting one vagus nerve (usually the left). As expected, merely cutting one vagus led to a fall in the rate of breathing and a small increase in the depth of breathing. Thereafter the second vagus nerve was cut about 5 sec after injection of phenyl diguanide, and the effect of the second vagotomy on the apnoea and the subsequent breaths were compared with the apnoea and rapid shallow breathing produced by phenyl diguanide in the absence of the second vagotomy. It should be noted that by 5–6 sec the main barrage of impulses produced by phenyl diguanide would have already passed up the vagus before it was cut (see Fig. 4).

The effects produced by the second vagotomy were variable. In two of the seven cats vagotomy did not reduce the apnoea produced by phenyl diguanide; in three there appeared a slowly increasing inspiratory activity after vagotomy. The fall in t_i after the apnoea was reduced in five cats after vagotomy; indeed in two of them t_i was even greater than control t_i (Fig. 8*B*). In four cats vagotomy did not alter the reduction in t_e; this was particularly striking in one cat (Fig. 8*A*) in which t_e fall was unchanged but t_i increased even with respect to control t_i (Fig. 8). In six out of seven cats vagotomy led to a smaller reduction in the amplitude of inspiration or the phrenic burst. In fact in one cat the mean frequency of discharge of the phrenic burst actually increased above the control value (Fig. 8*C*).

Injection of phenyl diguanide (45 μg/kg) given after bilateral vagotomy produced no effect on the fifteen cats tested. This confirms the observations of Dawes & Mott (1950).

In connexion with Fig. 8 it should be mentioned that in two cats bilateral vagotomy produced the expected marked fall in the rate of breathing and an increase in depth showing thereby that the accumulated doses of phenyl diguanide given prior to the bilateral vagotomy had no obvious influence on the effects of vagotomy. This conclusion receives support from the observed effects of cutting one vagus mentioned above.

Effects of repeated doses of phenyl diguanide. A second injection of phenyl diguanide given 5–10 sec after the first produced a much greater effect than the first. This was noted in all the ten cats examined. Indeed it was possible to elicit reflex effects by subthreshold doses given in this way. A third injection given in the same way after the second one produced effects similar to that of the second. These effects of the second (or third) injection can be attributed to a greater excitation of the J receptors by the second injection since in six J receptors it was found that the second injection given about 5–10 sec after the first produced a 2–17 times greater excitation than the first injection of phenyl diguanide. Indeed doses subthreshold for the receptor could yield a burst of impulses when a second dose was given within about 5 sec after the first injection. The enhanced effect of the second dose can be attributed to the build up of the concentration of phenyl diguanide at the receptor.

Effect of hypoxia and hypercapnia on the reflex effects of phenyl diguanide. The effect of hypoxia on the reflex respiratory responses following phenyl diguanide was examined in fifteen cats. In all of them reducing the inspired gas mixture to less than 11 % oxygen ($P_{a,O_2} < 45$ mmHg) reduced the duration of apnoea and the period of rapid shallow breathing. At P_{a,O_2} less than 35 mmHg the period of apnoea was replaced by brief periods of rapid shallow breathing (Fig. 9). This effect persisted after section of both carotid sinus nerves in four cats. However the aortic nerves remained

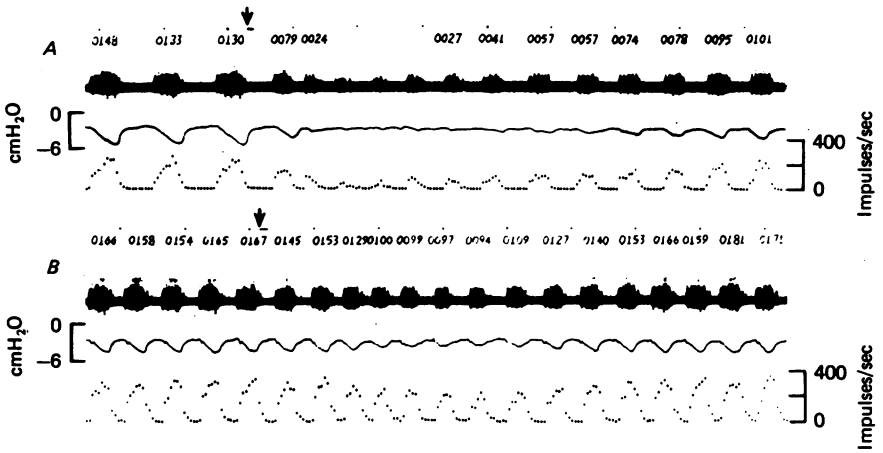

Fig. 9. Effect of hypoxia on the reflex effects of J receptors. *A*, the responses while the cat breathed 100 % O_2 (P_{a,O_2} = 403 mmHg). *B*, the responses while the cat breathed 8 % O_2 (P_{a,O_2} = 27 mmHg). Phenyl diguanide, 18 μg/kg, was injected at arrows in each record. From above downwards the traces, are, 2 sec time marks and injection signal on same trace, impulses in phrenic filament, intrathoracic pressure and the average frequency of discharge (calibration on the right). The shallow breathing produced by phenyl diaguanide in *A* persisted for over 6 min so that the amplitude of the breaths were still 50 % of the pre-injection value when 8 % O_2 gas mixture was turned on; record *B* was taken 1·5 min after that. Note total number of impulses in each breath in *B* cannot be compared with those in *A* (see Methods).

intact and therefore the influence of aortic chemoreceptors could not be excluded. The carotid sinus nerves were not cut in the experiment shown in Fig. 9.

Reduction in phrenic activity was also greatly diminished. This is well illustrated in Fig. 9*B* in which reducing the P_{a,O_2} to 27 mmHg not only abolished the 'apnoea' as indicated by the record of intrathoracic pressure when the cat was on oxygen (Fig. 9*A*) but it also greatly reduced the inhibition of phrenic motoneurones. In four cats the period of apnoea after phenyl diguanide increased while the cat breathed 100 % oxygen instead of air. However while on air the P_{a,O_2} was 50–65 mmHg in these cats apparently owing to the low respiratory rate and amplitude due to chloralose.

The above effects of hypoxia were not seen when the cat breathed hypercapnic gas mixtures (up to 4 % CO_2 in air).

It was established that the reduction in the reflex effects of J receptors at low arterial P_{O_2} was not due to reduction in the activity of J receptors, produced by phenyl diguanide, since in all five receptors tested the discharge following phenyl diguanide while the cat breathed 4·5–8 % O_2 in nitrogen was always greater than when it was breathing air (Fig. 10). This was not so when the cat breathed hypercapnic gas mixtures (Fig. 10). Indeed the total number of impulses while on hypoxic mixtures averaged 170 % of the value on air; the difference from control was significant ($P = < 0{\cdot}05$). The mean discharge of the same receptors following phenyl diguanide during hypercapnic mixtures was 95 % of control (not significantly different from control). Hypoxia (or hypercapnia) by itself had no excitatory effect on the receptors, all five remaining silent before injection of phenyl diguanide.

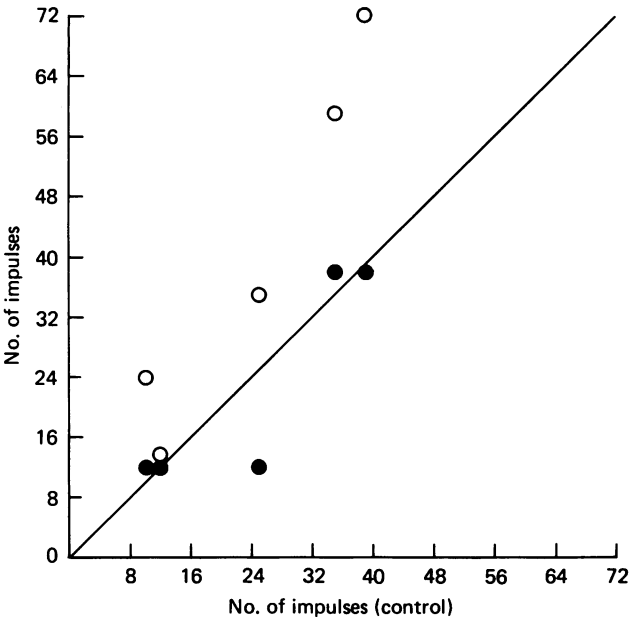

Fig. 10. Influence of hypoxia (○) and hypercapnia (●) on the responses of J receptors to phenyl diguanide 45 μg/kg. The abscissa represents the number of impulses generated in the receptors by phenyl diguanide while the cat breathed air. The ordinates represent the number of impulses generated by phenyl diguanide in the same receptors while the cat breathed hypoxic (4·5–8 % O_2) or hypercapnic (2·5 %–5·5 % CO_2) gas mixtures. In every case the receptors were silent before injection.

Reflex inhibition of muscles by J receptors. Injection of phenyl diguanide in doses of 12–45 μg/kg into the right atrium reduced the knee jerk by 50–75 % for at least $\frac{1}{2}$–1 min after block of impulses from cardiac receptors by xylocaine, so that left

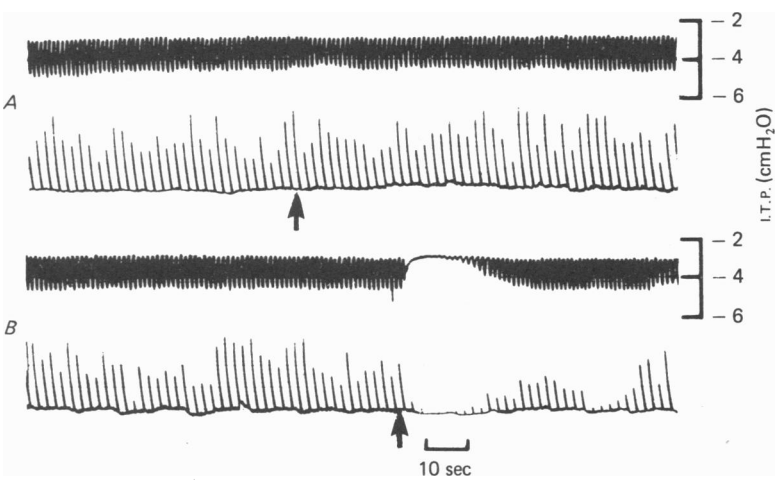

Fig. 11. Effect of injecting phenyl diguanide, 18 μg/kg into the left atrium (*A*) and the right atrium (*B*) at arrows after blocking impulses from cardiac receptors with 2 % xylocaine on respiration and the knee jerk. Note absence of any effect on injection into the left atrium and that the marked inhibition of the knee jerk lasted for 50 sec following injection into the right atrium.

atrial injections had no effect (Fig. 11). In some cats even small doses of phenyl diguanide abolished the knee jerk for some time (Fig. 11). Such responses were obtained repeatedly in the same cat under appropriate conditions of anaesthesia. These observations support the earlier conclusion that stimulation of J receptors leads to reflex inhibition of somatic muscles (Deshpande & Devanandan, 1970; Paintal, 1970; Kalia, 1973). However, in two cats it was also observed that injection of phenyl diguanide in doses as low as 10 μg/kg into the left atrium led to inhibition of the knee jerk thereby indicating that it is necessary to block the cardiac receptors if one wishes to study the reflex somatic effects of J receptors only.

Reflex inhibition of the knee jerk by stimulation of J receptors was observed in six out of ten cats in which there were marked respiratory reflex effects, i.e. apnoea lasting more than 10 sec followed by rapid shallow breathing. Depth of anaesthesia appeared to be an important factor; increasing the depth either reduced or abolished the inhibition of the knee jerk while the respiratory effects of phenyl diguanide injections persisted.

DISCUSSION

The most noteworthy conclusion of this investigation is that levels of activity in J receptors (produced by 12–18 μg phenyl diguanide/kg) equivalent to those generated by 133 % increase in blood flow through the lungs can produce profound respiratory reflex effects and inhibit somatic muscles. Such increase in blood flow could, under natural conditions, be produced by moderate exercise since Elkins & Milnor (1971) found that mean pulmonary blood flow increased by 105 % when dogs were made to run at a speed of 10·5 km/hr and Vatner & Boettcher (1978) observed an increase of 402 % in the cardiac output of dogs during severe exercise, i.e. running at a speed of 40 km/hr. Thus the role of J receptors in normal activity in man and animals acquires considerable significance not only at high altitudes as pointed out earlier (Paintal, 1969) but also at sea level. It is of even greater significance in clinical conditions, e.g. in patients with already congested lungs. However in exercise one should keep in mind that several factors are involved and the effects of the J receptors can be overshadowed by some of them, e.g. psychological factors and increased central drive to both respiratory and limb muscles.

As shown in Table 1 the mean pulmonary artery pressure increased by an average of 54 % when blood flow increased by 133 %. This order of increase is to be expected since Elkins & Milnor (1971) recorded an increase of 54 % when pulmonary blood flow increased by 105 % in their dogs during moderate exercise.

Earlier observations showed that the average activity in J receptors during severe pulmonary congestion leading to pulmonary oedema was 7·5 impulses/sec (Paintal, 1969). If this is taken to be maximum activity that can be generated during pulmonary congestion then it would appear that there is a nearly ten-fold range of activity between that produced by increasing blood flow by 133 % and that occurring during severe congestion leading to pulmonary oedema. The activity generated by 45 μg phenyl diguanide/kg (i.e. about 150 μg in averaged-sized cats) is therefore well within this range since, as shown in Fig. 5, the average frequency of the barrage generated by this dose of phenyl diguanide is about 4·8 impulses/sec. Thus apart from

the influence by activity from cardiac receptors (see below) the earlier observations of various investigators on reflex effects of J receptors using doses of this order acquire considerable significance and their relevance is not limited only to conditions leading to pulmonary oedema (cf. Paintal, 1973*a*).

The second important point that has emerged is that it is essential to block impulses from cardiac receptors if one wishes to study only the reflex effects of J receptors by injecting phenyl diguanide because stimulation of cardiac and other receptors without stimulating J receptors (i.e. by injecting phenyl diguanide into the left atrium) always produces some respiratory reflex effects which can be quite considerable in some experiments (e.g. Fig. 6*A*) and can also produce reflex inhibition of muscles in some experiments. Thus if one wishes to study the reflex effects of J receptors on muscle reflexes it is necessary to ensure that no reflex inhibition of muscles occurs on injections of phenyl diguanide into the left atrium with or without blocking the cardiac receptors. Since this was not done in the earlier studies (Deshpande & Devanandan, 1970; Kalia, 1973; Ahluwalia, Devanandan & Shukla, 1977; Rao and Devanandan, 1977) it is possible that the observations reported in them may need quantitative re-evaluation. However, it is certain that the conclusions reported in the above papers are qualitatively valid since inhibition of the knee jerk by stimulation of J receptors was seen in the present experiments after ensuring that left atrial injections of phenyl diguanide did not inhibit the knee jerk.

Re-evaluation of earlier observation of respiratory reflex effects beyond 5 sec following injections of phenyl diguanide into the right atrium will also be necessary. However since the latency for stimulation of the receptors supplied by arterial blood following right atrial injection is more than 4 sec (A. Anand & A. S. Paintal, unpublished observations) it follows that the earlier conclusions relating to apnoea and rapid shallow breathing limited to 4 sec of injection (Paintal, 1955) are quite valid.

It is reasonable to assume that selective stimulation of J receptors can be achieved by limiting the doses of phenyl diguanide to less than 30 μg/kg and simultaneously blocking the cardiac receptors with xylocaine because the doses required to stimulate aortic chemoreceptors (Paintal, 1967) and gastro-intestinal receptors are much higher – of the order of 60 μg/kg when given into the superior vena cava (Paintal, 1954). An additional advantage of xylocaine block is that the reflex effects of J receptors can be examined in the absense of marked reflex bradycardia. However this can also be achieved by injecting atropine and so if left atrial injections of phenyl diguanide only produce insignificant reflex effects one can attribute the major reflex effects by right atrial injections to J receptors.

The mechanisms responsible for reflex effects following injection of phenyl diguanide into the left atrium are as yet unknown. However since the reflex effects are blocked by intra-pericardial injections of xylocaine the receptors concerned must be located in the ventricles or atria such as those that are known to be stimulated by phenyl diguanide (Paintal, 1973*b*).

The third important point is that the central effects of J receptors can continue without intact vagi since cutting the vagus after injection of phenyl diguanide did not qualitatively alter the respiratory reflex effects of phenyl diguanide in a few cats, i.e. the reduction in t_i and t_e and respiratory amplitude occurred in the same way after cutting the single intact vagus (about 5 sec after injection of phenyl diguanide) as it

did with the single vagus intact. Thus the inputs from pulmonary stretch receptors do not play a dominant role in determining the reflex effects of J receptors. However it is also certain that the influence of vagal inputs is important because in certain other experiments cutting the vagus increased t_i and the activity of the phrenic (Fig. 8). These experiments showed that the effects on t_i and t_e could be dissociated (Fig. 8).

The results have shown that an input from J receptors can have a direct inhibitory effect on inspiration-excitatory mechanisms, i.e. on t_i and phrenic activity which is not surprising. However it is also certain that it can have a direct inhibitory effect on expiration-excitatory mechanisms because a reduction in t_e occurred as a first effect on several occasions (e.g. Fig. 9*A*). This cannot be a result of indirect inhibition, of the expiration-excitatory mechanisms, through facilitation of inspiratory mechanisms, because the breath following the reduced t_e (first effect) was always shorter and shallower (phrenic activity reduced, Fig. 9*A*). Thus it would be justifiable to conclude that J receptors produce their effects by directly inhibiting both expiration and inspiration-excitatory mechanisms. It is possible that the inhibition of one mechanism (e.g. expiratory) could be facilitated reciprocally through excitation of the other, but this needs to be established by more experiments.

The fourth point of significance is that reducing the arterial P_{O_2} reduces the respiratory reflex effects of J receptors in spite of 70 % increase in J receptor input produced by phenyl diguanide. At P_{O_2} less than 45 mmHg the apnoea produced by phenyl diguanide is reduced and at P_{O_2} less than 35 mmHg the apnoea may be replaced by rapid shallow breathing. Similar effects are not produced by hypercapnia and the effects of hypoxia occur after section of both carotid sinus nerves, thereby showing that the reduction of the reflex effects of J receptors by hypoxia is not due to increased inputs from chemoreceptors; it is therefore a central effect. It may be presumed that the respiratory sensations of J receptors (Jain, Subramaniam, Julka & Guz, 1972) may be similarly affected, i.e. the sensations may be reduced during hypoxia in spite of unchanged or even increased inputs from J receptors, a conclusion that may be of special significance at high altitudes.

The effects of hyperoxia on the reflex respiratory effects of J receptors is uncertain because in those cats in which the period of apnoea increased while on oxygen, the arterial P_{O_2} while they breathed air was 50–60 mmHg, i.e. the cats were hypoxic to begin with before oxygen was turned on. It is possible that the same may have been the case in the experiments reported in a preliminary communication by Wellhöner (1961).

The central mechanisms responsible for the respiratory and somatic reflex effects of J receptors are, as yet, largely unknown. However, the complexity of the central mechanisms involved is obvious from the varied effects produced by them. Apart from the central mechanisms responsible for the bradycardia which have not been studied in this investigation it is clear that it is possible to block the pathways responsible for the inhibition of muscles by increasing the depth of anaesthesia without blocking the respiratory reflexes. Moreover t_i and t_e can vary independently of each other and the influence of the vagal input (presumably mainly from pulmonary stretch receptors) on the mechanisms determining t_i and t_e is also variable from one experiment to the next. Indeed the only consistent reflex effect of J receptors is the reduction in the total number and mean frequency of phrenic impulses in

each breath. The reduction in the mean frequency stands in contrast to the observations of Winning & Widdicombe (1976) on single phrenic fibres. While considering the difference in the observations one should keep in mind that the observations of Winning & Widdicombe were made during reflexly produced marked hypotension and bradycardia which were absent or small in the present experiments owing to block of vagal efferent fibres by xylocaine.

Finally the fact that apnoea and rapid shallow breathing persist after cutting the second vagus nerve (first already cut) about 5 sec after injection of phenyl diguanide is noteworthy and is consistent with observations showing that the inhibition of the knee jerk (Kalia & Koepchen, 1974) and expiratory neurone activity (Koepchen, Kalia, Sommer & Klüssendorf, 1977) persists for long periods in the same way. Thus it is clear that unlike the inhibition by pulmonary stretch receptors which lasts only so long as the input from them is present the input from the J receptors has prolonged central effects. This shows further the complexity of the central mechanisms.

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