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THE RESPONSE OF PULMONARY AND CARDIOVASCULAR VAGAL RECEPTORS TO CERTAIN DRUGS

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Although much is already known about the reflex effects and receptor areas of veratrine (Bezold & Hirt, 1867; Jarisch & Richter, 1939; Krayer & Acheson, 1946; Dawes, 1947; Jarisch & Zotterman, 1948), isothiourea derivatives and the aromatic guanidines (Dawes & Fastier, 1950; Dawes & Mott, 1950; Dawes, Mott & Widdicombe, 1951), a knowledge of the afferent mechanisms concerned is largely obscure. Dawes *et al.* (1951) suggested that pulmonary vascular afferent fibres (Whitteridge, 1948) might be concerned in the inhibition of respiration following the intravenous injection of phenyldiguanide in the cat. At that time it was not known that these receptors were located in the right and left atria of the heart (Paintal, 1953*a*).

Two types of atrial receptors have been described (Paintal, 1953*b*): type A which gives a presystolic train of impulses in time with the *a* wave of the venous pressure curve, and type B which responds to changes in atrial filling and gives a late systolic train of impulses.

In this paper, the effects of veratrine, phenyldiguanide and pituitrin on the impulses in various vagal afferent fibres have been studied.

METHODS

The experiments were done on cats anaesthetized with chloralose (80 mg/kg). Single units were dissected and their action potentials recorded as already described (Paintal, 1953*c*). The right intra-atrial pressure was recorded with a semi-rigid catheter passed down the external jugular vein and connected to a saline-filled capacitance manometer. The intra-pleural pressure was recorded with a mirror membrane manometer.

To study the influence of the drugs on the vagal afferents single units were dissected and after identification a suitable dose of a drug was injected intravenously into a femoral vein while the activity of the fibre was recorded. Five to fifteen minutes were allowed to pass before another drug or another dose of the same drug was injected. The usual doses of the different drugs dissolved in 0·9% (w/v) NaCl solution were: veratrine, 1–7 mg; phenyldiguanide, 100 μg; pituitrin

0·5 ml. (Parke Davis 10 units/ml.). A control observation was made each time by a separate injection of 1 ml. of 0·9% NaCl solution.

During the early part of this investigation, it was found that injection of phenyldiguanide aroused activity in a previously inactive fibre with a conduction velocity of about 5 m/sec. In subsequent experiments the units sensitive to phenyldiguanide were isolated in the following way: after a strand had been dissected the vagal trunk was stimulated and the compound action potential examined for evidence of a slow component with a conduction velocity of below 15 m/sec. When such a component was present, phenyldiguanide was injected and the response noted on the cathode-ray tube. If an increase in base-line noise occurred, the strand was subdivided and the procedure repeated until a record of a single unit was obtained.

RESULTS

Phenyldiguanide

Phenyldiguanide when injected intravenously reduced the activity in slowly adapting pulmonary stretch fibres. This was associated with the inhibition of respiration produced by the drug phenyldiguanide; it did not produce increased activity in any stretch fibre. These observations are in agreement with those of Dawes *et al.* (1951). There was no significant effect on rapidly adapting stretch fibres (Knowlton & Larrabee, 1946) or on fibres firing only on deflation.

A marked decrease in activity of four depressor fibres was observed. This occurred about 3–4 sec after injection and was due presumably to the lowering of systemic blood pressure that has been shown to occur (Dawes *et al.* 1951). The discharge usually returned to its original level 30–60 sec later.

No significant change was seen in the activity of six right atrial type A fibres with repeated injections of phenyldiguanide. On occasions a small initial increase in the number of impulses per cardiac cycle associated with a slight rise in intra-atrial pressure was observed. No significant increase in the activity of three right atrial type B and one left atrial type B fibre was seen, although the inhibition of respiration and bradycardia were marked each time. As with the type A receptors and for the same reason, there was a small initial increase in the number of impulses in the type B fibres.

On nine occasions in seven cats phenyldiguanide stimulated to activity receptors that were previously quiescent (Fig. 1). All were characterized by a sudden outburst of activity 2–4 sec after injection of the drug. Activity was usually maximal in another 4–5 sec and died away after 10–25 sec. The degree of respiratory inhibition appeared to be related to the activity of these fibres. The response could be repeated several times and showed no significant diminution with subsequent injections. The conduction velocities of these fibres have been determined and the mean is about 6 m/sec (Paintal, 1953*c*).

Although many kinds of stimuli have been tried, no others have yet been found to produce any activity in units responsive to phenyldiguanide. Among the stimuli tested and found to be ineffective were (1) gradual or rapid inflations of the lungs, (2) suction of air from the trachea, (3) administration of

100% nitrogen or a mixture of 90% oxygen + 10% carbon dioxide for 2 min by a respiration pump, (4) intra-tracheal inhalation of ammonia, (5) the intravenous injection of a 1·3% (w/v) solution of $NaHCO_3$ to see if a change in the pH of the blood had any effect. In one case trichlorethylene administered by a respiratory pump also failed to arouse activity in the fibre. The effect of ammonia was tried as it had been suggested from a consideration of the structure of the amidines and their strongly basic nature that ammonia might resemble them in its action (Dawes, 1951).

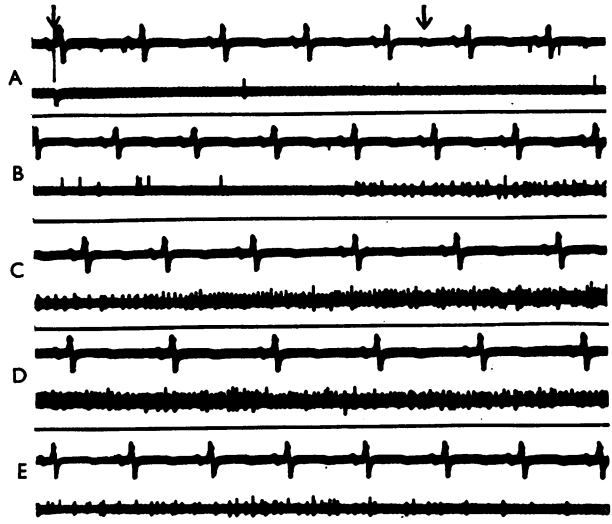

Fig. 1. Vagal afferent fibre stimulated by phenyldiguanide. A, B, C and D are continuous records, E is 9 sec later. From above downwards in each are e.c.g. and vagal impulses. 100 μg was injected intravenously between arrows in A.

The possibility that the phenyldiguanide sensitive endings might be located in the pleura was considered, and to test this 500 μg of phenyldiguanide was injected into the pleural cavity of one cat. No alteration in respiration or the heart rate was observed while an intravenous injection of 100 μg of the drug produced the usual marked bradycardia and respiratory inhibition.

Not all slowly conducting fibres show alterations in activity after the injection of phenyldiguanide; in groups of slowly conducting fibres (2–18 m/sec) it was often found that some remained inactive after this drug had been injected.

Veratrine

An intravenous injection of veratrine produces in pulmonary stretch fibres a high frequency continuous discharge, an observation in agreement with those of Dawes *et al.* (1951). The effect of veratrine was tried on three right atrial type A, one right atrial type B and three left atrial type B fibres. In none of

these was the activity altered significantly, although there was always a marked respiratory inhibition and bradycardia accompanying the injection. In one left atrial type B fibre the activity was increased (preceded by a decrease) after the period of respiratory inhibition and bradycardia had ended. This was probably not due to sensitization of the receptors but to greater left atrial filling. In one type A right atrial fibre the patterns of discharge altered (Fig. 2) as auricular fibrillation occurred after injection of veratrine.

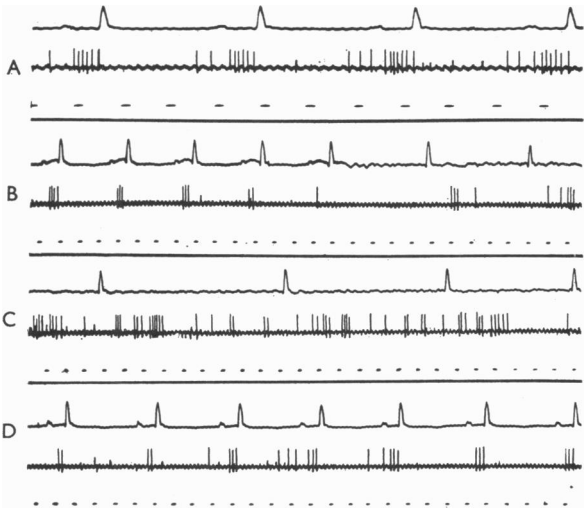

Fig. 2. A right atrial type A fibre. A is a normal record before injection of 10 mg veratrine intravenously; B, 5 sec after injection; C, 1½ min after injection and D, 6 min after injection. From above downwards in each are e.c.g., impulses in a fibre, time in $\frac{1}{10}$ sec. In B the moment auricular fibrillation occurs can be seen in the e.c.g. There is no increase in discharge after veratrine. Note the changes in pattern of discharge during auricular fibrillation. The e.c.g. and pattern of discharge are normal again in D.

Pituitrin

The effect of pituitrin was tried on two right atrial type A fibres, four right atrial type B, two left atrial type B, and one depressor fibre. In all there was a marked increase in fibre activity which was usually five to ten times the initial level.

In the right atrial fibres (Fig. 3) the increase in activity was associated with a rise in the right atrial pressure. This was probably a back pressure effect due to the marked rise in pulmonary arterial pressure which occurs with injection of such doses (0·5 ml.) of pituitrin. This latter fact was observed in two experiments in which the effect of pituitrin was tried on a constant volume inflow left lung perfusion of the cat. The relation of effective venous pressure to the number of impulses per cardiac cycle has been plotted in Fig. 4, which shows that the increased discharge can be explained entirely by the increased

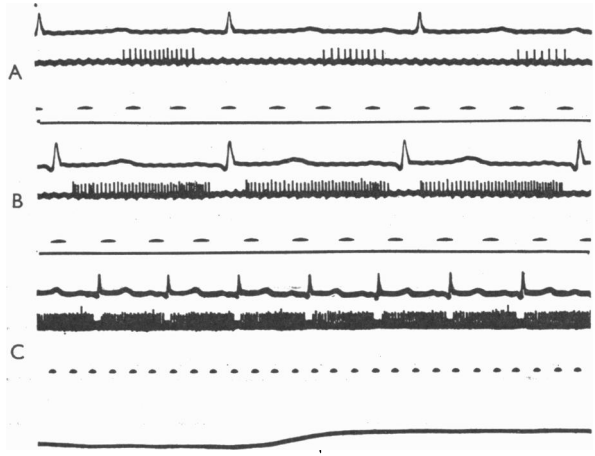

Fig. 3. Type B right atrial fibre. A is a normal record; B, after intravenous injection of 0·5 ml. pituitrin. C, taken at slow camera speed, shows the absence of obvious difference in discharge during inspiration and expiration. From above downwards in each are e.c.g., impulses in a fibre, time in $\frac{1}{10}$ sec. In C the lowest trace is a record of the intrapleural pressure, inspiration downwards.

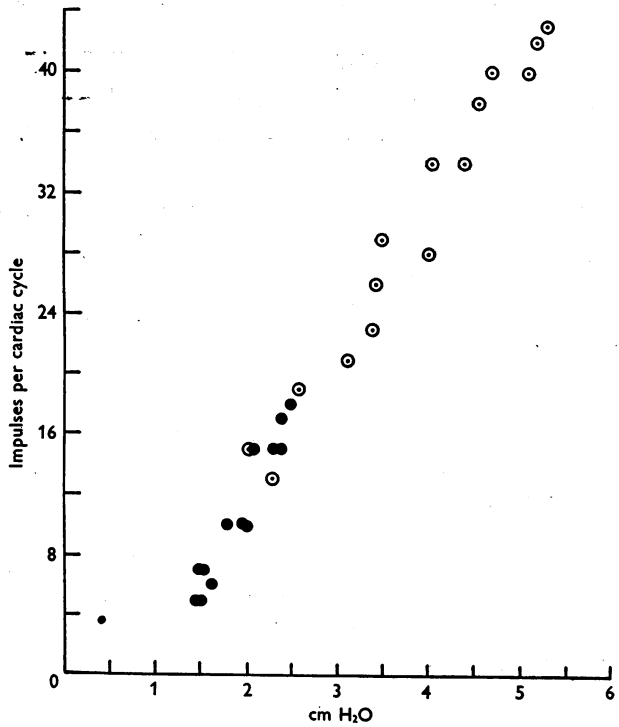

Fig. 4. Relation of effective venous pressure and number of impulses per cardiac cycle in a right atrial type B fibre. Solid circles represent values before intravenous injection of 0·5 ml. pituitrin; open circles after injection. Graph shows absence of sensitization of receptor by pituitrin.

pressure resulting from the increased filling of the atrium on the assumption that atrial filling and pressure are related.

During the action of pituitrin the pattern of the discharge usually alters considerably and may on occasion become continuous. The type A fibres often temporarily lose their *a* volley of impulses for no obvious reason—the discharge then occurring entirely in the systolic period. During increased activity in the type B fibres, the volley of impulses may begin very early in systole, often immediately after the QRS complex of the e.c.g. (Fig. 3). The fibres may then develop temporarily a *c* volley of impulses in time with the *c* wave of the venous pressure curve. In one fibre the discharge developed a mid-systolic silent interval. During the period of increased activity the respiratory variations in the discharge are small (Fig. 3), occasionally there are none.

Injection of pituitrin caused a large increase in activity in a depressor fibre—the discharge becoming continuous with a superimposed cardiac rhythm for about 30 sec. This was probably due to the increase in systemic blood pressure. However, unlike the atrial receptors, the pattern of discharge was not much altered after the period of continuous discharge had ceased.

In the left atrial type B fibres a similar increase in activity occurs. This is probably due to a rise in systemic blood pressure causing a back pressure effect analogous to the conditions obtained in the right atrium. Fig. 5 shows the effect of pituitrin on a left atrial fibre. In this the respiratory variations which are opposite to those occurring on the right side are accentuated. It is not known whether these fibres are sensitized or not, as there was no record of the left atrial pressure, but from the character of the changes it is presumed that they were not.

Pituitrin does not affect pulmonary stretch fibres and it therefore provides a reliable way of distinguishing between atrial receptors and stretch fibres with a cardiac rhythm.

DISCUSSION

In the investigation of the effects of drugs on receptors, it is always desirable to record the physical or chemical factors which normally affect that ending and simultaneously the frequency of the impulses set up. Unfortunately this was not possible for the stretch and arterial pressor receptors described here. It was done for some of the atrial receptors, and it was then easy to distinguish between effects of a drug on the sensitivity of an ending, and effects of a drug on the physical environment of that ending. It could not be done with the small fibres whose endings were sensitive to phenyldiguanide since their natural stimulus is unknown.

It seems certain that phenyldiguanide, in doses sufficient to produce respiratory inhibition and bradycardia, does not stimulate or sensitize the known respiratory and cardiovascular receptors. The suggestion of Dawes *et al.* (1951) that the pulmonary vascular receptors (atrial type B, Paintal, 1953*b*) might

be the ones concerned with the amidine reflex is therefore not confirmed. This suggestion was made from the fact that the blocking temperature of these fibres (Torrance & Whitteridge, 1948) was the same as the temperature at which the reflex respiratory inhibition produced by the drug was blocked, namely 3° C. From a consideration of the conduction velocities of various vagal afferents (Paintal, 1953*c*) it is likely that several different types of vagal

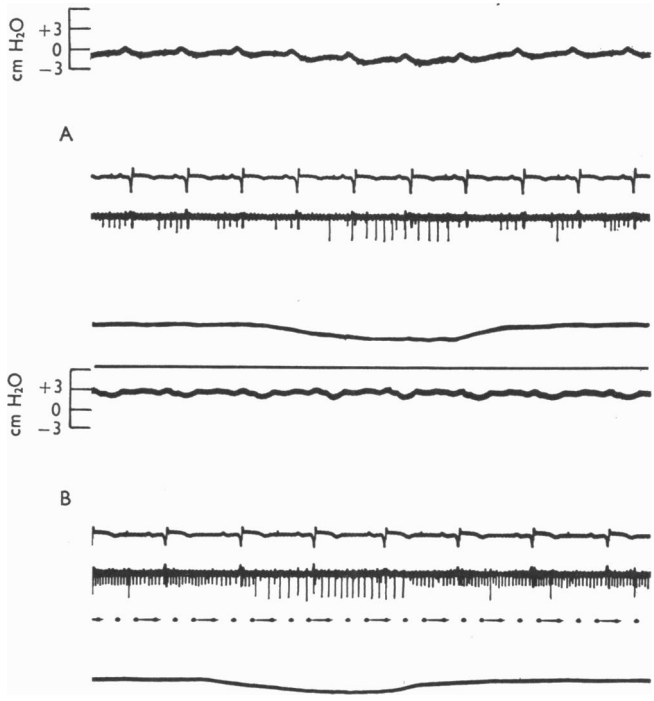

Fig. 5. Effect of pituitrin on a left-atrial receptor (small spike). A, before and B, after intravenous injection of 0·5 ml. pituitrin. From above downwards, right venous pressure record, e.c.g., impulses in fibres, intrapleural pressure (inspiration downwards). In B there are time marks in $\frac{1}{10}$ sec. Note that activity in the left atrial fibre is maximal during expiration. Also note the rise in right venous pressure after injection. The pulmonary stretch fibre (large spike) is unaffected by the drug.

afferents probably possess similar blocking temperatures, and it would therefore appear difficult to prove from differential blocking experiments alone that only one particular type of afferent was concerned in the production of reflex respiratory inhibition. The fact that phenyldiguanide produces different reflex effects in different animals suggests the possibility that various reflex mechanisms may be involved (Dawes, Mott & Widdicombe, 1951, 1952).

It is certain that phenyldiguanide stimulates the receptors of certain slowly conducting fibres (mean conduction velocity of 6 m/sec) that were previously

inactive. It is also definite that it does not increase the activity in all slowly conducting fibres, but it is not certain whether all the fibres roused to activity are functionally the same. However, all these fibres have in common the fact that they are normally inactive and that they are unaffected by inflation or deflation of the lungs, or by administration of nitrogen or carbon dioxide. These phenyldiguanide-sensitive receptors could be amongst those concerned in the inhibition of respiration as their mean conduction velocity of 6 m/sec might correspond to the blocking temperature of the reflex which is 3° C. They are probably not concerned in the depressor reflexes produced by the drug from the heart and lungs, for as shown by Dawes *et al.* (1951) these responses are blocked at higher temperatures; their afferent fibres remain unidentified so far.

The normal functions of the phenyldiguanide-sensitive receptors remain to be determined. If they are concerned with the reflex inhibition of respiration they are probably located in the lungs as the experiments of Dawes *et al.* would suggest. Their existence would have remained unnoticed without the use of drugs and this technique might therefore reveal the presence of normally quiescent fibres in other situations. It is believed that there are probably several types of afferent fibres normally quiescent in the vagus and the use of other types of drugs might reveal their existence.

The observation of Dawes *et al.* (1951) that veratrine sensitizes or excites the pulmonary stretch receptors is confirmed, but it does not affect the right and left atrial receptors of both types A and B. No fibres of the type described by Jarisch & Zotterman (1948) as responding to veratrine were encountered; possibly a further search would yield evidence of their existence. The afferent fibres concerned in the Bezold reflex remain unknown.

The use of pituitrin has revealed the fact that the pattern of discharge in atrial receptors of both types A and B may change radically and assume unusual forms. It would therefore seem necessary to exercise caution in the interpretation of unusual patterns of discharges with a cardiac rhythm. Pituitrin provides an easy way of increasing the activity of these receptors although the increase is secondary to changes in atrial filling and atrial pressure.

SUMMARY

1. The responses in various pulmonary and cardiovascular afferent fibres to intravenous injections of phenyldiguanide, veratrine and pituitrin have been studied and it has been shown that:

(*a*) Phenyldiguanide in doses sufficient to cause inhibition of respiration and bradycardia does not stimulate or sensitize slowly or rapidly adapting pulmonary stretch receptors, arterial pressure receptors, right and left atrial receptors of both types A and B, and fibres firing only on suction of air from the trachea.

(*b*) Phenyldiguanide stimulates endings of certain fibres which are normally

quiescent and which have a mean conduction velocity of 6 m/sec. These receptors are not affected by inflation or deflation of the lungs or by administration of 100% nitrogen or a mixture of 90% oxygen and 10% carbon dioxide.

(*c*) Veratrine stimulates the slowly adapting pulmonary stretch receptors but not the aortic or atrial receptors.

(*d*) Injection of pituitrin gives rise to greatly increased activity in atrial receptors. This is associated with a rise in intra-atrial pressure.

2. It is possible that the phenyldiguanide-sensitive receptors may be concerned in the reflex inhibition of respiration produced by the drug.

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