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CIRCULATORY AND RESPIRATORY REFLEXES CAUSED BY AROMATIC GUANIDINES

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A number of more or less complicated chemical compounds are known to produce, on intravenous injection, a reflex fall of blood pressure and heart rate by an action on nerve-endings in the heart (the Bezold reflex). These include the veratrum alkaloids (von Bezold and Hirt, 1867 ; Jarisch and Richter, 1939 ; Krayer and Acheson, 1946 ; Dawes, 1947), an extract of mistletoe (Jarisch, 1941 ; Job, 1943), and adenosine triphosphate (Emmelin and Feldberg, 1948). In addition Dawes and Feldberg (1949) have shown that horse serum elicits a similar reflex in the cat, and that this is in part responsible for the " Brodie phenomenon " (Brodie, 1900), the name given to the consequent fall of blood pressure and heart rate.

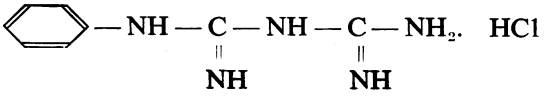
In the course of an investigation of various substances which cause a fall of blood pressure by the liberation of histamine (MacIntosh and Paton, 1949), Dr. W. Paton observed that certain diguanides had an action superficially resembling that of the veratrum alkaloids, in that the fall of blood pressure and heart rate was abolished by cutting the vagi. He very kindly sent us eight of these substances, which had been prepared by Dr. H. King, with the suggestion that they might also cause the Bezold reflex. This was the starting point for the investigations described in the following paper.

METHODS

The majority of the experiments were performed on cats under chloralose or pentobarbitone (nembutal) anaesthesia ; a few rabbits under urethane anaesthesia were also used. For the localization of the site of action two preparations were used which have been described in a previous paper (Dawes, 1947). In order to facilitate injection into one of the main coronary arteries, this was connected to one carotid by a rubber tube and cannula introduced through the left subclavian artery, and passed down the ascending aorta into the coronary orifice ; the cat was given heparin to prevent clotting. For injection into the right or left ventricles while normal respiration was maintained, the heart was " exteriorized " by an incision betweeen two ribs on the left side of the chest, and the pleura reconstituted by sewing the pericardium to the edges of the wound. Respiration was recorded by a modification of Gaddum's method (1941).

RESULTS

The simplest of the eight compounds with which the investigation started was phenyldiguanide hydrochloride.



Forty-two related substances have since been investigated, and their activity has been compared with that of phenyldiguanide, which was adopted as a standard. The twenty-four compounds which were inactive, or had very little activity, were not studied in detail, and the subsequent description of the properties of this group of substances applies, therefore, only to those compounds (Table I), closely related to phenyldiguanide, which possess considerable activity in causing a fall of blood pressure and heart rate on intravenous injection.

Examples of the fall of blood pressure and heart rate caused by the injection of phenyldiguanide and *o*-chlorophenyldiguanide into cats can be seen in Figs. 1, 2, 4, and 5. The fall is very sudden and even startling; at times indeed it seemed doubtful if the circulation would recover. Yet it did recover, even though the heart rate for a few beats might be reduced to one-quarter or less of the initial rate, and though the blood pressure fell to 30 mm. of mercury (Fig. 1). The depressor response did not usually last more than a minute, often considerably less. It could be obtained repeatedly in the majority of animals, even at frequent intervals over a period of hours; it was therefore possible to estimate the relative activity of a number of such substances. In this respect phenyldiguanide differs from the veratrum alkaloids, repeated injections of which lead to a total failure of the response. Occasionally an animal was encountered which showed this phenomenon with phenyldiguanide, but to a much smaller degree.

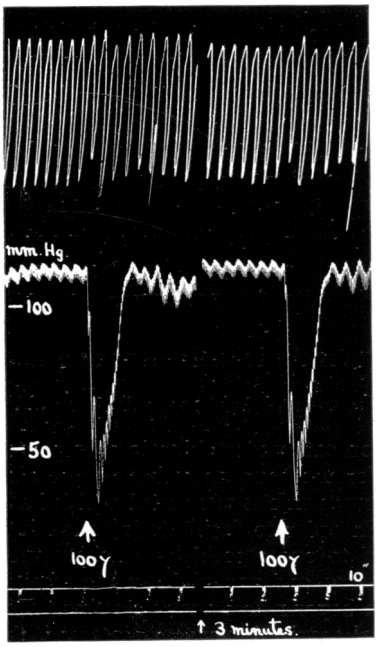


FIG. 1.—Cat, 2.6 kg.; chloralose anaesthesia. Above, respiration; below, blood pressure. At each arrow 100 μg. phenyldiguanide was injected into the jugular vein.

In some animals the fall of blood pressure and heart rate caused by the injection of phenyldiguanide or its analogues was accompanied by little or no change in respiration (Fig. 1); in others there was slowing or an abrupt cessation of respiration (Fig. 2). But in all animals inhibition of respiration was seen when the dose was increased. It has even been observed in cats in which there was little or no change of blood pressure or heart rate. The change in respiration is therefore independent of the change in the circulation, nor is it likely to originate from a chemical or physico-chemical action on the same structure. Yet both circulatory and respiratory effects are abolished by cutting the vagi in the cat.

In the rabbit phenyldiguanide also causes a fall of blood pressure and heart rate, accompanied by an inhibition of respiration. The fall of heart rate and the inhibition of respiration are abolished by cutting the vagi, but the fall of blood pressure, though commonly reduced, does not usually disappear altogether.

Before discussing the circulatory and respiratory responses in more detail there is one further variable which must be described. At the outset we were anxious to obtain a rough estimate of the minimal effective dose of phenyldiguanide necessary to cause a fall of blood pressure and heart rate, for comparison with veratridine. This was finally estimated to be about 10 μg./kg. in the cat, though exceptionally a cat would respond to as little as 5 μg./kg. ; only 1–2 μg./kg. veratridine were required to produce a similar response. There were, however, a few cats which did not appear to respond at all, even to an injection of 500 μg. phenyldiguanide. These cats were apparently healthy and in good condition. It was also noticed that even in those cats in which phenyldiguanide or its analogues did cause a large fall of blood pressure and heart rate at the beginning of the experiment, after an hour or two the responses became larger and more regular in size and duration. Hitherto we had used for anaesthesia an ethyl-chloride–ether induction, followed by chloralose 0.08 g./kg. or pentobarbitone 25 mg./kg. intravenously. We had the impression, largely derived from previous work with veratridine, that of these two anaesthetics chloralose was preferable, since the initial blood pressure was higher, the falls were larger, and there was no need to give an additional dose of anaesthetic for six hours or more, indeed for the entire duration of the experiment. But since it appeared possible that the anaesthetic might influence the response to a greater extent than hitherto supposed, we reduced the quantity of chloralose to 0.06 g./kg. This led to much better results. Although the responses still tended to increase during the first hour of anaesthesia, from that time we encountered no animal which failed altogether to respond during the first hour, and the animals were maintained under efficient anaesthesia for five hours or more. The effect of a small dose of pentobarbitone on the reflexes is illustrated in Fig. 2. In the experiment from which this tracing is taken, the cat, weighing 3.8 kg., had been anaesthetized with chloralose (0.06 g./kg.) and a number of equal responses to 100 μg. *o*-chlorophenyldiguanide obtained, of which only the last one is shown, at 12.35 p.m. Between this and the next injection, at 12.40, a dose of 3 mg. pentobarbitone per kg. was administered intravenously (this would correspond to barely one-eighth

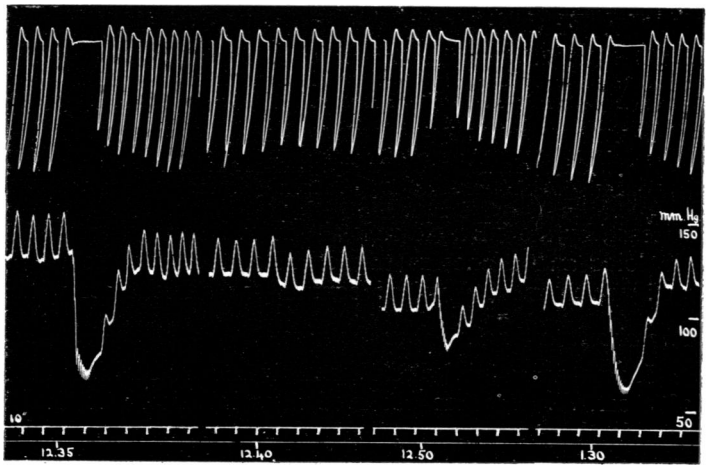


FIG. 2.—Cat, 3.8 kg.; chloralose anaesthesia, 60 mg./kg. Above, respiration; below, blood pressure. At each signal mark 100 μg. *o*-chlorophenyldiguanide was injected intravenously. Between 12.35 and 12.40 p.m., a dose of pentobarbitone (3 mg./kg.) was injected.

of the normal intravenous anaesthetic dose). Both cardiovascular and respiratory reflexes were totally abolished, although the effect of the barbiturate on blood pressure and respiration was barely perceptible. After fifteen minutes the reflex responses could be elicited again, though full recovery took nearly an hour. (The large respiratory variation in blood pressure in this animal was spontaneous, and is not attributable to the resistance of the respiratory valves.) This susceptibility to anaesthetics will explain our earlier failures to obtain the reflexes in some animals.

The circulatory reflex from the heart

If 100 μg. phenyldiguanide or one of its analogues is injected with a fine needle directly into the cavities of the right or left ventricle of a cat with its chest opened and artificially respired, the fall of blood pressure and heart rate begins with dramatic suddenness after a latent period of from 2–5 seconds. But the reflex is not obtained when the injection is made into the aorta. It therefore seems highly probable that it is, in part at least, due to an action on structures within the area of distribution of the coronary arteries. That this is so can be seen from experiments of which Fig. 3 is an illustration. An injection of 10 μg. *p*-methyl-phenyldiguanide into the cavity of the right atrium had no action on blood pressure or heart rate. When the same dose was injected into the left coronary artery (perfused from the left carotid) the typical fall of blood pressure and heart rate was observed. Similar observations have been made with phenyldiguanide and a number of the other more active compounds, and there can be little doubt that they all cause a fall of blood pressure and heart rate, in part at least, by the "Bezold reflex." It will be recollected that, as with the veratrum alkaloids and adenosine triphosphate, the response is abolished by cutting the vagi, which must therefore contain the afferent nerve fibres.

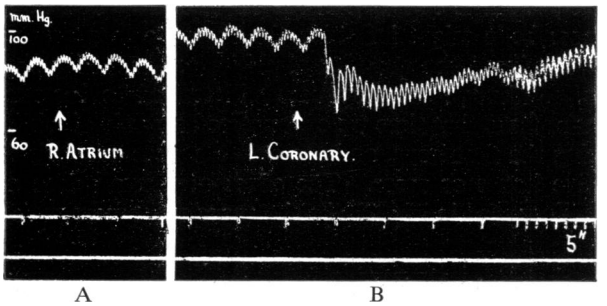


FIG. 3.—Cat, 2.9 kg.; chloralose anaesthesia; chest opened along the midline, artificial respiration; left coronary artery connected to the left carotid by a cannula and rubber tube as described elsewhere. Record of the blood pressure. At A 10 μg. *p*-methyl-phenyldiguanide was injected into the cavity of the right atrium; at B the same quantity was injected into the left coronary artery.

The circulatory reflex from the lungs

If veratridine is injected alternately into the cavities of the right and left ventricles of a cat, the response observed after injection into the right ventricle is always considerably smaller, and has a longer latent period (about 8 seconds) than on injection into the left ventricle (2.5–4.0 seconds). From this it has been deduced that, though there are receptors in the lungs of the dog which can be stimulated by veratridine to cause a fall of blood pressure and heart rate, they are either absent or of very little importance in the cat. With phenyldiguanide and its homologues, however, the findings are altogether different. If a cat is prepared with an open

thorax, under artificial respiration, and with cannulae tied into the two atrial appendages, it is relatively easy to make repeated injections at nearly the same speed into either atrium. In such experiments, an illustration of which is to be seen in the upper part of Fig. 4, not only is the latent period between injection into either atrium

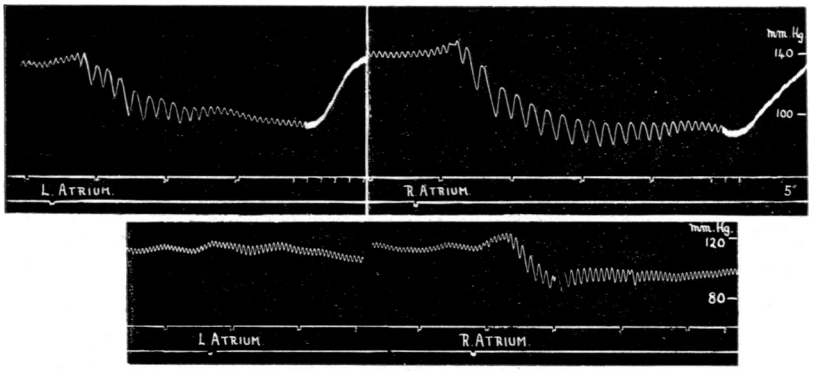


FIG. 4.—Cat, 2.4 kg.; chloralose anaesthesia; chest opened along the midline, artificial respiration; cannulae for injection tied into both atrial appendages. Record of the blood pressure. At each signal 100 μg. phenyldiguanide was injected into the atria as indicated. Between the upper and lower parts of the tracing all accessible nerve fibres running in the atrio-ventricular grooves were divided.

and the fall of blood pressure or heart rate approximately the same (3 seconds), but the response is usually greater, and often considerably greater on injection into the right atrium. A similar result is obtained when phenyldiguanide is injected by a fine needle directly into the pulmonary artery. This can only mean that there must be receptors in the lungs which are sensitive to phenyldiguanide, as well as those in the heart.

It seemed very likely that the afferent fibres from the lungs would run along the pulmonary veins and join the vagi at a different level from the afferent fibres from the heart. So it should be possible to cut one group of fibres and leave the other intact. Preliminary attempts to achieve this result by stripping out all the nerves and ganglia betweeen the pulmonary artery and the arch of the aorta, and the nerves running along the first cm. of the left coronary artery, were unsuccessful. But a certain degree of success was achieved when the epicardium and nerves running in or athwart the atrio-ventricular grooves (in which the coronary arteries lie) were divided. Between the upper and lower parts of Fig. 4 this dissection was performed, and it can be seen that while the effect of injecting 100 μg. phenyldiguanide into the left atrium (reflex from *heart only*) was virtually abolished, the effect of injection into the right atrium (reflexes from *heart and lungs*) was only reduced. This experiment provides additional support for the view that a considerable proportion of the depressor response caused by phenyldiguanide comes from reflexes, the receptors for which are in the lungs.

In the experiment illustrated, it would appear, if the relative effects of injection into the left atrium before division of the nerves and into the right atrium after their division are compared, that the heart and lung receptors contributed very

roughly half each to the total response. This would agree well with the rough conclusions drawn from a comparison of the proportionate effects of injecting veratridine and phenyldiguanide into right and left atria.

The respiratory reflex

Veratridine has been shown to cause a slowing or transitory stoppage of respiration by a reflex originating from the lungs (Dawes, 1947). It seemed possible that the inhibition of respiration caused by phenyldiguanide might originate in the same way. It was found that while injection into the cavity of the right ventricle or pulmonary artery of the "exteriorized heart" caused an immediate inhibition of respiration, a similar injection into the left ventricle had little or no action (Fig. 5). The respiratory response usually began slightly earlier than the cardiovascular, and the Figure shows once again the greater cardiovascular response on injection into the right side of the heart.

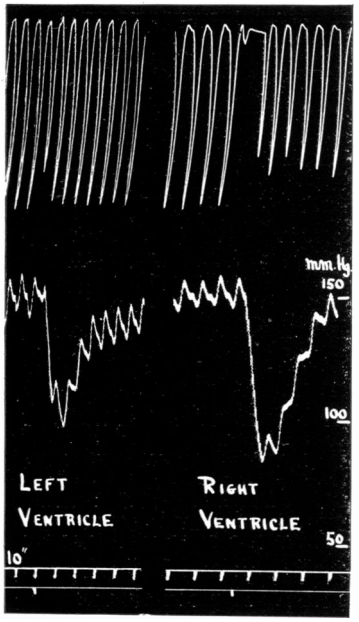


FIG. 5.—Cat, 3.8 kg.; chloralose anaesthesia; heart "exteriorized" as described elsewhere, natural respiration. Records: above of the respiration, below of the blood pressure. At each signal 100 μg. *o*-chlorophenyldiguanide was injected into the cavities indicated by a fine needle.

Other actions of phenyldiguanide

An intravenous injection of 50–100 μg. phenyldiguanide usually causes a considerable fall of blood pressure and heart rate in a cat, and some depression of respiration. If the vagi are cut this dose has no cardiovascular or respiratory action, and it is not until a dose some 20–40 times larger is injected that any further action on the circulation is observed; 2–5 mg. phenyldiguanide injected into a cat under chloralose anaesthesia with the vagi cut causes a rise of blood pressure, and in the spinal cat this rise may be as much as 80 mm. of mercury, lasting a few minutes. Subsequent injections are less and less effective. These responses are reminiscent of those observed with so many amines, amidines, and *iso*thioureas in large doses and do not call for any special comment.

In the rabbit it has been observed that a small dose of phenyldiguanide may cause a reduced fall of blood pressure even after the vagi are cut. In part this may be due to a direct action on the heart; the force of contraction of the Langendorff preparation of the rabbit's heart is reduced by the injection of 200 μg. or more of phenyldiguanide (it may be remarked that this represents a very high concentration of the drug). Similar observations have also been made on the isolated auricle of the rabbit. The Langendorff preparation of the cat's heart is somewhat less sensitive to phenyldiguanide. This may be a partial explanation of the difference in the effect of vagotomy in the rabbit and cat, but there are other possibilities which have not yet been investigated (relatively

greater vasodilatation in the rabbit either directly or perhaps by liberation of histamine).

These observations call attention to the specificity of phenyldiguanide and its active homologues in exciting these particular cardiovascular and respiratory reflexes. Since in the cat injection into the arch of the aorta failed to cause a change of blood pressure, heart rate, or respiration in doses of 100 μg. or more, it was improbable that the carotid sinus, carotid body, or medullary centres were susceptible to any considerable extent. An attempt to excite these structures by injecting retrograde from the lingual artery, with or without occlusion of the cephalic end of the external carotid, was unsuccessful.

Phenyldiguanide also had no considerable action on either neuromuscular or ganglionic transmission. The contractions of the gastrocnemius of a cat under chloralose anaesthesia were recorded during stimulation of the sciatic nerve by square current pulses of short duration ; the injection of up to 2 mg. phenyldiguanide via the external iliac artery of the opposite side did not alter the twitch tension. Similarly intravenous injection of up to 2 mg. phenyldiguanide into a cat under chloralose (the vagi being cut to eliminate circulatory disturbances) did not alter the contractions of the nictitating membrane in response to stimulation of the cervical sympathetic ; 10 mg. phenyldiguanide caused a sustained contraction of the nictitating membrane lasting more than an hour, and not affected by removal of the superior cervical ganglion. In contrast to the veratrum alkaloids phenyldiguanide had no taste when placed on the tongue.

The relation between chemical structure and activity

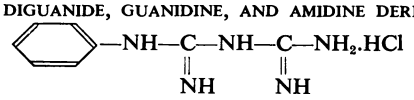
The relative simplicity of the phenyldiguanide molecule and its close structural resemblance to compounds occurring naturally in living organisms aroused our curiosity about its chemical analogues. A number of compounds were tested for their activity in causing a fall of blood pressure and heart rate, and in slowing or stopping respiration, in cats under chloralose anaesthesia ; a dose was then found which matched the effect of phenyldiguanide on the blood pressure. Phenyldiguanide was commonly used in a dose of 50–100 μg. ; where a new compound had no effect in a dose of 1–2 mg. (i.e., when its activity was less than one-twentieth that of phenyldiguanide) it has been classified as inactive. Assays were carried out on five cats at the most, for each compound, and the less active compounds were tested on fewer cats. The figures given in the following Tables are intended only to give a general idea of the order of activity.

From Table I it will be seen that small changes in the substituents on the benzene ring of phenyldiguanide produce a tenfold variation in activity. The most active compounds are those with a methyl or chlorine atom in the *ortho* position. Phenylguanidine possesses considerably less activity than phenyldiguanide, but *p*-chlorophenylguanidine is as active as phenyldiguanide. The three amidines, phenylacetamidine, propionamidine, and butyramidine, were very much less active.

So far as our rather crude method of recording the respiration permitted, we also concluded that in the compounds listed in Table I change in activity on the cardiovascular system was accompanied by an approximately equal change in the capacity to elicit the respiratory reflex.

TABLE I

ACTIVE DIGUANIDE, GUANIDINE, AND AMIDINE DERIVATIVES



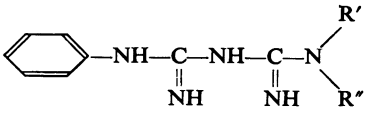
Substituent groups	Depressor activity in terms of phenyldiguanide
None	1.0
para-Cl	1.5
ortho-Cl	2.5
para-Methyl	0.3
meta-Methyl	1.0
ortho-Methyl	1.5
meta-Dimethyl	0.6
para : *meta*-Dimethyl	0.8
para-Ethyl	0.2
para-Methoxy	0.5
ortho-Methoxy	0.2
1 : 1-Diphenyldiguanide, HCl	0.05
Phenylguanidine, HNO_3	0.15
p-Chlorophenylguanidine, HNO_3	1.0
o-Chlorophenylguanidine, HNO_3	0.1
p-Chlorophenyl-N^2-methylguanidine, HCl	0.1
Phenylacetamidine, HNO_3	0.05
β-Phenylpropionamidine benzenesulphonate	0.15
γ-Phenylbutyramidine benzenesulphonate	0.10

The introduction of an aliphatic group in place of one of the hydrogen atoms on the terminal nitrogen of the diguanide chain abolishes activity. Six such compounds were tested, including paludrine (Table II). From Table III it will be seen that the substitution of a phenyl or *p*-chlorophenyl radical for one of the hydrogens on the terminal nitrogen of the side chain also abolishes activity.

In very large doses phenylacetamidine does cause a fall of blood pressure—cf. Goodwin and Marshall (1945). This may be accompanied by a small fall of heart

TABLE II

DIGUANIDE DERIVATIVES WITH LESS THAN $\frac{1}{20}$TH THE ACTIVITY OF PHENYLDIGUANIDE



Substituent groups	R′	R″
para-Cl	CH_3	H
para-Cl	CH_3	CH_3
para-Cl	C_2H_5	C_2H_5
para-Cl	nC_3H_7	H
para-Cl	$isoC_3H_7$	H
ortho-Cl	$isoC_3H_7$	H

.TABLE III

DIGUANIDE AND AMIDINE DERIVATIVES WITH LESS THAN $\frac{1}{20}$TH THE ACTIVITY OF PHENYLDIGUANIDE

Diguanide	1 : 5-diphenyldiguanide
1-methyldiguanide	1 : 5-di(*p*-chlorophenyl) diguanide
1 : 1-dimethyldiguanide	N-methyl-phenylacetamidine
1-*iso*propyldiguanide	*p*-aminobenzamidine
1 : 2-trimethylenediguanide	*p*-chlorobenzamidine
1 : 1-pentamethylenediguanide	*p*-hydroxyphenylbenzamidine
1-cyclohexyldiguanide	Spermine, HCl
1-benzyldiguanide	Dodecamethylene diguanide HCl (synthalin)
1-phenyl-1-methyldiguanide	2-pyridylbenzamidine

rate but in two animals was not abolished by vagotomy, whereas the depressor action of phenyldiguanide was abolished. Since such large doses of phenylacetamidine were required the analysis of its mode of action was not pursued further. The range of compounds in Table III which possess little or no activity help to limit still further the known structural requirements for activity.

Discussion

Phenyldiguanide and the other compounds listed in Table I possess unusual properties. They share with the veratrum alkaloids, mistletoe extract, adenosine triphosphate (ATP), and an unidentified substance or substances from serum, the ability to cause a reflex fall of blood pressure and heart rate by an action on receptors in the heart. Like serum, they also excite a similar depressor reflex from receptors in the lungs of the cat: this latter property veratridine does not possess to any measurable degree. In addition there is the respiratory inhibition, shared by veratridine and not yet thoroughly investigated in serum or ATP. But apart from these three reflexes, the phenyldiguanides are singularly inactive. The veratrum alkaloids have been investigated extensively for nearly a century, and are known to have extraordinarily fascinating actions on nerve, the neuromuscular and ganglionic junctions, and an action which in some ways resembles that of digitalis upon the heart. It is possible that the cardiovascular and respiratory reflexes caused by the veratrum alkaloids are but a special instance of their capacity to induce repetitive firing in nerve. Yet they too show to nearly the same degree the considerable specificity for the two reflexes (depressor from the heart; respiratory inhibition from the lungs) which von Bezold and Hirt (1867) first described. Thus after vagotomy the dose of veratridine has to be increased ten- or twenty-fold before any further pharmacological action is observed. On the other hand according to Emmelin and Feldberg (1948) ATP in the dose needed to cause a fall of blood pressure and heart rate (0.2–0.4 mg.) also causes a constriction of the pulmonary vessels of the cat, and a slightly larger dose gives rise to muscular contractions, mainly of central origin. We are left wondering why these three distinct reflexes should together be so sensitive to the phenyldiguanides and their analogues, and what there can be in common between phenyldiguanide, the veratrum alkaloids, and the unknown substance in serum.

From the physiological point of view by far the most interesting questions raised are the natural purposes of the two cardiovascular reflexes, if indeed they do play a normal part in physiological function. The cardiac depressor or Bezold reflex has been discussed in a previous paper (Dawes, 1947). Since that time Jarisch and

Zottermann (1948) have added to our knowledge of it, and it seems possible that the natural stimulus may be a rise of pressure in the left ventricle, though there is by no means a direct proof of this hypothesis.

The other depressor reflex, which originates in the lungs, is of equal interest. We were surprised to find that the injection of a chemical compound could cause such a large fall of blood pressure and heart rate by an action on receptors in this area. It is true that veratridine had previously been shown to have a similar action in the perfused lungs of dogs (Dawes, 1947), but this was a small effect and it was not seen in cats. However, the depressor reflex from the lungs caused by the injection of serum in cats (Brodie, 1900 ; Dawes and Feldberg, 1949) is very similar ; it has been attributed by Gilding and Nutt (1944) to a protein of the albumin class. And as long ago as 1900 Brodie and Russell observed that inhalation of bromine, or stimulation of the central ends of the pulmonary branches of the vagus, caused a fall of blood pressure and heart rate. Some light may be shed on the problem by the observations of Schwiegk (1935) and Daly, Ludany, Todd, and Verney (1937), who described a reflex slowing of the heart when the pressure in the pulmonary circulation is raised. Whitteridge (1948) has recently found a number of vagal fibres which from their behaviour he believes may arise from endings on the small vessels of the lung. The central connexion of these fibres is unknown, and it has not been found possible to stimulate the central ends of these fibres without stimulating many others. The evidence from these different experimental studies appears to be converging, and it is possible that phenyldiguanide and its homologues might excite, directly or indirectly, the fibres described by Whitteridge.

An additional point of interest is the importance of the depth of anaesthesia for the detection of these three reflexes (the two depressor and the respiratory). In our experience a very small excess of either chloralose or pentobarbitone abolishes them (Fig. 2). The animals showed considerable variation in their behaviour under chloralose in particular. Gilding and Nutt (1944) observed on injection of stored plasma into cats peristalsis, micturition, defaecation, vomiting, and muscular contractions of a pattern similar to that seen with adenosine triphosphate (ATP) and described further by Emmelin and Feldberg (1948). It is evident from their work that these reactions to stored plasma and ATP are more readily seen in decerebrate than in chloralose cats and that this difference can be ascribed to the effect of the anaesthetic. Since Emmelin and Feldberg (1948) and Dawes and Feldberg (1949) showed that ATP and horse serum elicit the Bezold and other vagal reflexes in cats it seems probable that all these reflexes are similarly susceptible to a small increase in the depth of anaesthesia. It is interesting to speculate on the application of these observations to anaesthesia in man ; the reflexes in question can be abolished without any change in the level of the general blood pressure, and unless specific search is made for these reflexes one would not know from casual observation that they had been abolished as the depth of anaesthesia is increased.

Discussions of the relation between structure and function are not always profitable. In this series of compounds it would appear that an aromatic group at one end of the molecule is required for high activity, since the first seven compounds in Table III are inactive. At the other end the side chain may contain

an unsubstituted guanidine group ; a number of other variants remain to be explored. Certain comparisons may be made at this point ; for instance, phenylguanidine is active, whereas phenylacetamidine has very much less activity and may even produce its depressor action in a different way. The benzamidine derivatives tested were also inactive. On the other hand it was surprising to find that 1-benzyldiguanide and 1-methyl-1-phenyldiguanide were inactive. Substitution in the benzene ring can also cause a considerable alteration of activity. Thus *ortho*-substitution of a methyl group or chlorine atom increased the activity of phenyldiguanide, whereas *ortho*-substitution of chlorine in phenylguanidine reduced activity. This latter observation has been checked several times by direct comparison of the two compounds. On the other hand *para*-substitution of a chlorine atom in phenylguanidine causes a far greater increase in activity than in phenyldiguanide.

Up till last year the veratrum alkaloids were the only pure chemical compounds known to excite the Bezold reflex. Their structure was not known exactly, but it was believed to contain a six-membered ring of a complexity unlikely to occur naturally in the animal body. Since then both ATP and phenyldiguanide have been found to cause reflex disturbances of the circulation and respiration. ATP certainly occurs in the body, and the phenyldiguanide and phenylguanidine molecules are sufficiently simple to raise the possibility that they or one of their homologues may also do so. Yet there is not sufficient evidence to decide whether the reflexes discussed above are elicited by a direct action of phenyldiguanide and its homologues on nerve endings, or by an action on other structures which causes a physical change in the vicinity of the nerve endings. Until this point is finally settled any discussion of the possible part played by guanidine derivatives in the normal physiological function of these reflexes would be premature.

Summary

A number of phenyl-guanidine and -diguanide derivatives cause, on intravenous injection, a considerable fall of blood pressure and heart rate in cats, sometimes accompanied by a transient inhibition of respiration. These phenomena are abolished by cutting the vagi, and are attributed to three reflexes:

(1) A fall of blood pressure and heart rate due to a reflex from the heart itself, since it could be elicited by injection of a small dose into the left coronary artery.

(2) A fall of blood pressure and heart rate due to a reflex from the lungs.

(3) An inhibition of respiration due to a reflex from the lungs.

These reflexes are very readily abolished by a slight increase in the depth of anaesthesia, using either chloralose or pentobarbitone. The substances investigated showed a remarkable specificity in exciting these three reflexes, and were otherwise relatively inactive. Forty-two related compounds have been studied in an investigation of the structural requirements for this type of activity.

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References

Bezold, A. von, and Hirt, L. (1867). *Unters. physiol. Lab. Würzburg*, **1**, 73.
Brodie, T. G. (1900). *J. Physiol.*, **26**, 48.
Brodie, T. G., and Russell, A. E. (1900). *J. Physiol.*, **26**, 92.
Daly, I. de B., Ludany, G. V., Todd, A., and Verney, E. B. (1937). *Quart. J. exp. Physiol.*, **27**, 123.
Dawes, G. S. (1947). *J. Pharmacol.*, **89**, 325.
Dawes, G. S., and Feldberg, W. (1949). *J. Physiol.*, **108**, 362.
Emmelin, N., and Feldberg, W. (1948). *Brit. J. Pharmacol.*, **3**, 273.
Gaddum, J. H. (1941). *J. Physiol.*, **99**, 257.
Gilding, H. P., and Nutt, M. E. (1944). *J. Physiol.*, **102**, 446.
Goodwin, L. G., and Marshall, P. B. (1945). *J. Pharmacol.*, **84**, 16.
Jarisch, A. (1941). *Arch. exp. Path. Pharmak.*, **197**, 266.
Jarisch, A., and Richter, H. (1939). *Arch. exp. Path. Pharmak.*, **193**, 347, 355.
Jarisch, A., and Zottermann, Y. (1948). *Acta physiol. scand.*, **16**, 31.
Job, C. (1943). *Arch. exp. Path. Pharmak.*, **202**, 633.
Krayer, O., and Acheson, G. H. (1946). *Physiol. Rev.*, **26**, 383.
MacIntosh, F. C., and Paton, W. D. M. (1949). *J. Physiol.*, **109**, 190.
Schwiegk, H. (1935). *Pflüg. Arch. ges. Physiol.*, **236**, 206.
Whitteridge, D. (1948). *J. Physiol.*, **107**, 496.