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PHARMACOLOGICAL PROPERTIES OF PHENYLDIGUANIDE AND OTHER AMIDINE DERIVATIVES IN RELATION TO THOSE OF 5-HYDROXYTRYPTAMINE

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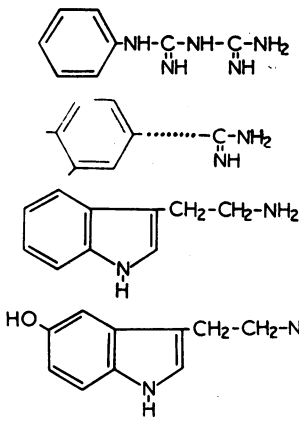
Cats in which the coronary and allied chemoreflexes could not be obtained with small intravenous doses of 5-hydroxytryptamine were insensitive also to phenyldiguanide. In cats which responded to phenyldiguanide with reflex falls of blood pressure and heart rate, abolished by vagotomy, the effects of graded doses (5 to 150 μg./kg.) of phenyldiguanide bore a striking resemblance to those produced initially by 5-hydroxytryptamine in somewhat smaller doses. Differences in the cardiovascular responses to the two drugs are attributed to additional (non-reflex) actions of 5-hydroxytryptamine. The reflex actions of both drugs were blocked reversibly also by 2-naphthylguanidine (500 μg.). Certain other drugs (bufotenine, procaine, *S*-decylisothiourea) antagonized the depressor action of phenyldiguanide as well as the reflex depressor action of 5-hydroxytryptamine. Like 5-hydroxytryptamine, phenyldiguanide and certain other amidine derivatives caused pain when applied to the base of blisters in human subjects. Unlike 5-hydroxytryptamine, phenyldiguanide did not constrict perfused rat blood vessels or increase the tone of the rat fundal strip preparation of Vane (1957). Phenyldiguanide did not affect the sensitivity of these smooth muscle preparations to 5-hydroxytryptamine, but other amidine derivatives proved to be moderately strong antagonists of the vasoconstrictor actions of 5-hydroxytryptamine and of adrenaline. Unlike 5-hydroxytryptamine, phenyldiguanide did not produce gastric haemorrhage in the mouse. Phenyldiguanide did not prolong chloral hydrate sleeping time in mice by the same mechanism as did 5-hydroxytryptamine. Phenyldiguanide was not highly toxic to mice (LD50 being 240 mg./kg.). It is concluded that phenyldiguanide and certain other amidine derivatives act on sensory receptors which respond to 5-hydroxytryptamine, but that they show little pharmacological resemblance to 5-hydroxytryptamine in other respects.

The chemoreflexes produced by phenyldiguanide (I) and other amidine derivatives were first studied by Dawes and Mott (1950), who concluded that these compounds caused the stimulation of certain sensory receptors in the cardio-pulmonary area. Dawes and Fastier (1950) tested about 100 substances related chemically to phenyldiguanide in the hope that the structural features of compounds found to act like phenyldiguanide might recall those of a substance which could be the natural stimulant of the receptors on which these drugs act. They found that nearly all the more potent compounds were based on the structure consisting of aromatic nucleus–short side chain–unsubstituted amidine group (II).

"Serotonin" had not been fully characterized at the time of this investigation; however, there was evidence that it was a tryptamine derivative. Since it was known that the intravenous injection of serum into cats can cause a reflex fall of blood pressure and heart rate (Brodie, 1900), tryptamine (III) was tested by Dawes and Fastier (1950) and was found to be inactive. However, 5-hydroxytryptamine (IV) produces chemoreflexes which closely resemble those produced by such amidine derivatives as phenyldiguanide (Page, 1952; Comroe, Van Lingen, Stroud and Roncoroni, 1953; Kottegoda and Mott, 1955). It was therefore suggested (Fastier, 1955) that such compounds as phenyldiguanide may resemble 5-hydroxytryptamine sufficiently closely in



chemical structure for them to be able to stimulate the same chemoreceptors. Evidence bearing on this hypothesis has been obtained in the investigation now to be described.

METHODS

Cats were anaesthetized with chloralose (60 to 70 mg./kg.). Blood pressure was recorded from a carotid artery. The method used for recording respiratory movement was essentially that of Gaddum (1941). Drug solutions were injected into a saphenous vein. Rabbits were anaesthetized with pentobarbitone sodium, guinea-pigs with urethane, and Australian possums (*Trichosurus vulpecula*) with chloralose.

Effects on cutaneous pain receptors in man were studied by the method of Armstrong, Dry, Keele, and Markham (1953). This involves raising a blister on the flexor surface of the forearm, cutting away the separated epidermis to expose the base of the blister, and recording the degree of discomfort estimated by the subject when various solutions are applied to the exposed blister base. A five-point scale was used, no pain being rated 0, slight pain 1, moderate pain 2, fairly severe pain 3, and very severe pain 4. The subject noted the degree of discomfort at 15 sec. intervals. Cantharidin was used as the blistering agent for all the experiments described. However, as one of us developed an extensive papular rash after the third application of cantharidin, we now use a pellet of CO_2 snow in place of cantharidin. Drugs were applied in neutral isotonic solution. The subject was not told what drugs were being tested.

In sleeping time experiments, mice in batches of 10 were given a subcutaneous injection of either an amidine derivative or saline 2 hr. before an intraperitoneal injection of chloral hydrate (250 mg./kg.). Mice were numbered so that individual sleeping times could be accurately assessed. The sleeping mice were kept in boxes at a temperature of 24 to 25°. A cross-over experiment was performed four days later, the mice which previously had been given the amidine derivative being given saline, and vice versa. The sleeping times of individual mice when given chloral hydrate alone were subtracted from their sleeping times when given chloral hydrate together with the amidine derivative. The logarithm of this difference was taken as the variate in statistical tests, since we found that the log. sleeping times of control mice were normally distributed whereas the sleeping times were positively skewed. The rectal temperatures of the mice were measured by inserting a glue-covered constantan-copper thermocouple into the rectum to a fixed distance.

Rat hindquarters were perfused at a constant rate with Ringer-Locke solution as described by Fastier and Smirk (1947). Effects of drugs on the fundal portion of the rat's stomach were studied following the technique of Vane (1957).

Compounds other than amidine derivatives were obtained from commercial sources. Most of the amidine derivatives were samples used in previous studies. Specimens of 1-naphthylguanidine, 1-naphthyldiguanide, and 2-naphthyldiguanide were synthesized for us by Mr. L. C. K. Wong. We are indebted to Professor Adrien Albert for the 2-naphthylguanidine.

RESULTS

Effects on the Circulatory System

Reflex Actions

It is now well established that the coronary and allied chemoreflexes are not elicited by phenyldiguanide in vagotomized animals. Even in animals with intact vagi, small intravenous doses of phenyldiguanide do not always elicit sharp falls of blood pressure and of heart rate. This led us to inquire whether those animals which responded atypically to phenyldiguanide would also do so to 5-hydroxytryptamine.

Cats.—Examination of the kymograph records of previous experiments had shown that the responses to phenyldiguanide and 5-hydroxytryptamine are very similar, considering the variation from cat to cat. Typical pairs of responses are illustrated in Fig. 1. As the variation in the course of a single experiment was often considerable, in all the pairs of responses shown the second drug was given within 5 to 8 min. of the first. Predominantly depressor responses were obtained with both phenyldiguanide and 5-hydroxytryptamine in 27 out of 32 cats, almost wholly pressor responses in three others. In two cats the responses to the two drugs differed much more than the paired responses shown in Fig. 1; whereas a small dose of phenyldiguanide produced slight falls of blood pressure, one of 5-hydroxytryptamine produced a slight rise.

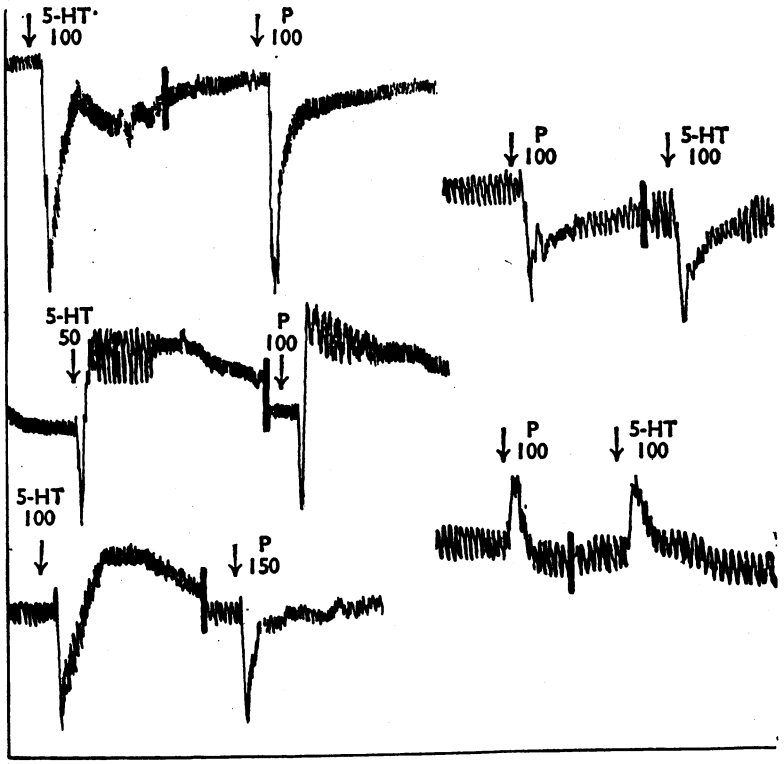


FIG. 1.—Facsimiles of kymograph records showing effects of small doses of 5-hydroxytryptamine (5-HT) and of phenyldiguanide (P) on the arterial blood pressure of anaesthetized cats. Note that the considerable variation from one cat to another in the response to 5-HT is reflected by a similar variation in the response to P. The numerals indicate the dose in μg.

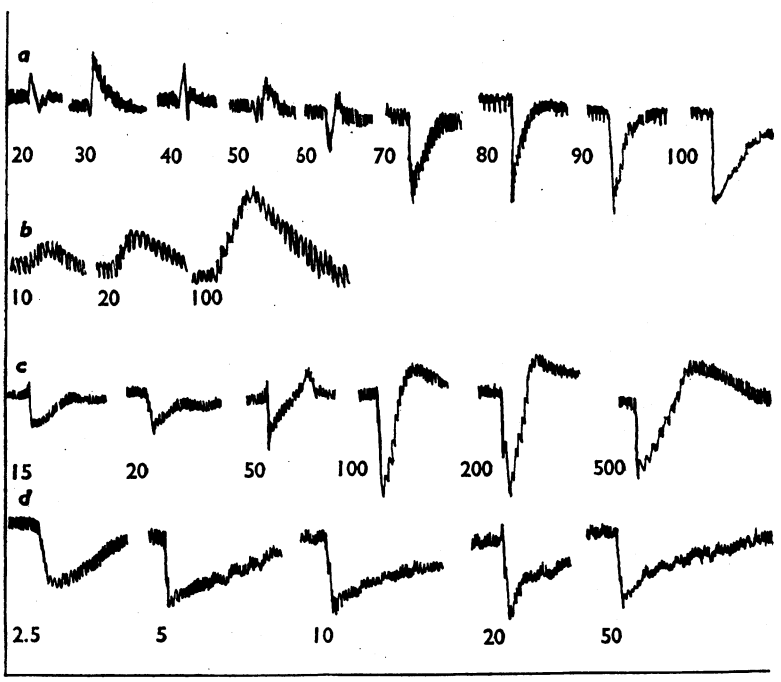


FIG. 2.—Facsimiles of kymograph records showing effects of 5-hydroxytryptamine in graded doses (indicated in μg. by the numerals) on the arterial blood pressure of four anaesthetized cats (*a*, *b*, *c*, and *d*).

In a further series of 18 experiments a wide range of doses was given. In 9 of these experiments doses from 1 to 500 μg. 5-hydroxytryptamine were given. There was a gradual transition from a small pressor to a large depressor response. Fig. 2*a* shows a typical series of responses. Pressor responses only were obtained in 4 out of the other 9 cats (Fig. 2*b*), depressor responses only in the remaining 5 (Figs. 2*c* and *d*).

A pressor element was much less evident in the responses to graded doses of phenyldiguanide. Differences in the cardiovascular effects of the two drugs could therefore be explained by supposing that 5-hydroxytryptamine exerts not only reflex actions practically identical with those of phenyldiguanide, but also an action not shared by phenyldiguanide. While the later phases of the response to 5-hydroxytryptamine differed substantially from those to phenyldiguanide in 12 cats, the initial phases of the responses were usually similar. After vagotomy, pressor responses were generally obtained with 5-hydroxytryptamine, but phenyldiguanide was almost without action on blood pressure.

Other Species. — Reflex depressor responses were readily obtained with both phenyldiguanide and 5-hydroxytryptamine in experiments on rabbits but not on guinea-pigs or Australian possums. The blood pressure of the latter was usually raised by 10 to 100 μg./kg. of 5-hydroxytryptamine, sometimes by more than 50 mm. of mercury, though only for a few minutes. Phenyldiguanide produced small rises of blood pressure in 4 out of 5 possums; the fifth animal responded to both phenyldiguanide and 5-hydroxytryptamine with falls of blood pressure. The pressor responses in other possums were sometimes preceded by slight falls of blood pressure and heart rate. Guinea-pigs resembled possums rather than cats and rabbits in their responses to both drugs.

Interaction with Other Drugs.—In experiments on cats anaesthetized with chloralose, other drugs were given to see if they selectively blocked the reflex depressor actions of 5-hydroxytryptamine and phenyldiguanide.

2-Naphthylguanidine was tested initially because of its chemical resemblance to *S*-2-(2′-naphthyl)ethylisothiourea, the most potent of the amidines examined by Dawes and Fastier (1950). Both 1- and 2-naphthylguanidine, as well as the corresponding diguanides, were found highly active in evoking chemoreflexes. The effects of 2-naphthylguanidine (100 to 200 μg.) were very similar to those produced by somewhat smaller

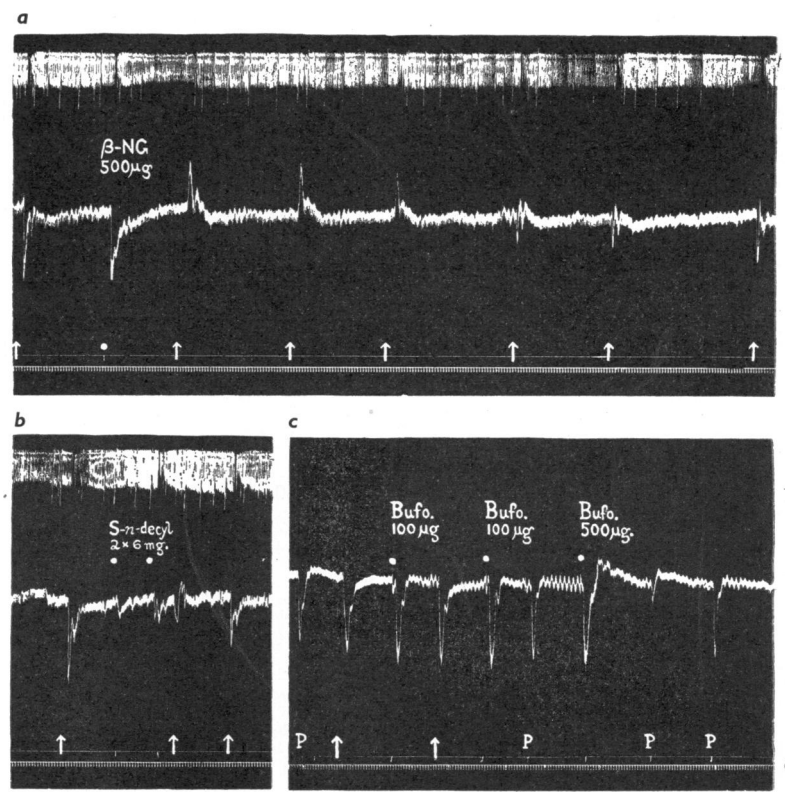


FIG. 3.—*a*, Anaesthetized cat, 6.7 kg. Records from above downwards, respiratory movements, arterial blood pressure (initial level 190 mm. of Hg) and time (10 sec.). Note temporary inhibition of reflex responses to 5-hydroxytryptamine given in 100 μg. doses at each arrow after administration of 500 μg. 2-naphthylguanidine (β-NG). Injections were made at 5 min. intervals. *b*, Continuation of the experiment illustrated in *a*. Note temporary inhibition of reflex effects of 5-hydroxytryptamine given at arrows by *S*-decylisothiourea hydrobromide. (S-*n*-decyl) in two doses of 6 mg. at white dots. *c*, Anaesthetized cat. Note in this experiment that bufotenine (Bufo.) in 100 μg. doses produces reflex falls of blood pressure which closely match those produced by 100 μg. doses of phenyldiguanide and 5-hydroxytryptamine (given at P and the arrows respectively), and that a large dose of bufotenine temporarily inhibits the reflex depressor action of phenyldiguanide.

doses of phenyldiguanide in the same cats. After a larger dose of 2-naphthylguanidine (500 μg.) the response of the cat to subsequent doses of phenyldiguanide or 5-hydroxytryptamine was greatly modified for some 10 to 30 min. Fig. 3 shows a typical result: whereas in untreated animals 5-hydroxytryptamine produced typical falls of blood pressure, after 2-naphthylguanidine it caused rises of blood pressure, the response gradually returning to normal as the effect of the guanidine wore off. The response to phenyldiguanide was greatly diminished or even abolished by 2-naphthylguanidine for some 10 to 20 min., but there was no reversal of the action of phenyldiguanide.

Other amidine derivatives, such as 2-naphthyldiguanide and *S*-decylisothiourea, resembled 2-naphthylguanidine in being able to inhibit reversibly the reflex depressor actions of 5-hydroxytryptamine and phenyldiguanide. This is shown for *S*-decylisothiourea in Fig. 3. Blocking of the depressor action of 5-hydroxytryptamine by preceding large doses (up to 1 mg.) of phenyldiguanide could not be clearly demonstrated. When phenyldiguanide and 5-hydroxytryptamine were given at intervals of 5 min. or more, there was no sign of interaction.

Bufotenine was tested like 2-naphthylguanidine for its ability to evoke chemoreflexes. Depressor effects obtained with bufotenine (50 or 100 μg.) in cats closely resembled those with 5-hydroxytryptamine in the same doses. These amounts of bufotenine did not alter the response to 5-hydroxytryptamine or phenyldiguanide given 5 min. later, but 500 μg. of bufotenine substantially diminished the falls of blood pressure obtained with 100 μg. of 5-hydroxytryptamine or phenyldiguanide, though only for 5 to 10 min. This is also shown in Fig. 3.

Procaine had little or no blocking action in doses of 1 to 3 mg./kg.; 10 to 20 mg./kg. abolished the fall of blood pressure obtained with 100 μg. of either 5-hydroxytryptamine or phenyldiguanide.

Adrenaline or *noradrenaline* was infused for 15 to 30 min. at intervals through a jugular vein while test doses of phenyldiguanide and 5-hydroxytryptamine were injected into the saphenous vein. The catechol amines usually raised arterial blood pressure by 50 mm. or more of mercury when given in saline at a rate of 8 to 20 μg./ml./min. In three cats, the depressor effects of phenyldiguanide and 5-hydroxytryptamine were enhanced during the first infusion, and in the remaining four cats they were not much affected. Different results were obtained when adrenaline or noradrenaline was infused for a second or third time, the depressor actions of phenyldiguanide and 5-hydroxytryptamine being either markedly reduced (3 cats) or entirely abolished (4 cats).

Iproniazid or *reserpine* was given to some cats before experiments, as it seemed possible that the response to injected 5-hydroxytryptamine might depend to a large extent upon the amount of endogenous 5-hydroxytryptamine present. Iproniazid is believed to delay the destruction of 5-hydroxytryptamine *in vivo* by inhibiting amine oxidase, and reserpine to deplete the body of 5-hydroxytryptamine and certain other amines. In 5 of 6 cats given two doses of iproniazid (50 mg./kg., the first dose 18 hr. and the second 1 hr. before the main experiment), small intravenous doses of 5-hydroxytryptamine and phenyldiguanide produced falls of blood pressure which did not differ from those obtained in untreated cats. One of the cats pre-treated with iproniazid responded with a rise of blood pressure to small doses of 5-hydroxytryptamine and phenyldiguanide. Pre-treatment with reserpine (2 mg./kg. intraperitoneally 18 hr. before the experiment) likewise failed to produce in anaesthetized cats reflex responses different to those seen in untreated animals.

Other drugs, such as methylamphetamine or pecazine (Pacatal, 10 mg.), temporarily inhibited the reflex depressor actions of 5-hydroxytryptamine and phenyldiguanide.

Lysergic acid diethylamide did not block the reflex depressor action of 5-hydroxytryptamine when given either shortly before it or at the same time in doses of up to 200 μg.

2-(Pentachlorophenoxy)ethylamine was tested because Woolley (1957) has shown that compounds of this type resemble 5-hydroxytryptamine in certain respects. Larger doses (5 or 7 mg.) produced sharp falls of blood pressure and temporary arrest of breathing. The amine did not antagonize the depressor action of 5-hydroxytryptamine to any noteworthy extent, and this action was only partly abolished by vagotomy. This action therefore appears to be due only in part to chemoreflexes.

Actions on Perfused Blood Vessels

In experiments on perfused hind-quarters of the rat, 5-hydroxytryptamine had about 1/10 of the vasoconstrictor activity of adrenaline. Whereas 0.2 μg. of adrenaline was generally sufficient to increase perfusion pressure temporarily by some 60 to 100 mm. Hg, 3 to 5 μg. of 5-hydroxytryptamine was needed to produce a comparable

effect. Phenyldiguanide had no effect on perfusion pressure when tested in doses of from 10 to 100 μg., and it did not modify the response of the perfused vessels to either 5-hydroxytryptamine or adrenaline. Like phenyldiguanide, many of the other amidine derivatives highly effective in evoking chemoreflexes had no effect on vessel tone in doses of up to 100 μg.; however, some of them differed from phenyldiguanide in that they strongly antagonized the vasoconstrictor action of 5-hydroxytryptamine. The active amidines included 2-naphthylguanidine, *S*-(3-phenylpropyl)-isothiourea, and the *o*- and *p*-chloro derivatives of *S*-benzylisothiourea. All these compounds antagonized the action of 5-hydroxytryptamine appreciably when given in a dose not more than 20 times that of 5-hydroxytryptamine, namely 50 μg. of amidine as against 5 μg. 5-hydroxytryptamine. However, the most potent of these compounds had still less than 1/100 of the activity of lysergic acid diethylamide as an antagonist of 5-hydroxytryptamine under the same conditions. Most of the amidines antagonized the vasoconstrictor action of adrenaline about as readily as that of 5-hydroxytryptamine. There was no correlation between potency in reflexly lowering blood pressure in cats and in antagonizing the vasoconstrictor action of 5-hydroxytryptamine.

Effects on the Respiratory System

Previous work, reviewed by Dawes and Comroe (1954), indicates that the effects of phenyldiguanide and 5-hydroxytryptamine on respiratory movement are chiefly due to chemoreflexes evoked by the stimulation of sensory endings in the cardio-pulmonary area. In the cat moderate doses (20 to 50 μg./kg.) usually cause transient apnoea followed by rapid shallow breathing. In other species both agents may produce somewhat different effects.

Their most usual effect in our experiments on guinea-pigs and possums was to inhibit breathing for a few seconds and then stimulate it markedly for several minutes. With these animals, as with cats, a fairly close similarity in the respiratory effects of the two drugs was noted. In support of the idea that they act on the same sensory endings when causing temporary apnoea in the cat is the observation that this effect is inhibited reversibly by 2-naphthylguanidine (see Fig. 3) and by *S*-decylisothiourea.

Effects on Cutaneous Pain Receptors

Armstrong *et al.* (1953) have shown that 5-hydroxytryptamine excites cutaneous pain receptors in man when applied in dilute solution to the exposed blister base. We have used their technique to see if phenyldiguanide and certain other amidine derivatives resemble 5-hydroxytryptamine in this respect.

Experiments were performed on four subjects. They reacted to most drugs in a similar fashion, but several differences were noticed. Thus one subject experienced no pain with acetylcholine even when the concentration was increased to 10^{-3} g./ml. By giving various drug solutions repeatedly, it was possible to gauge the ability of the subject to discriminate between different solutions. Saline or a very dilute solution of a potent drug never produced more than a threshold

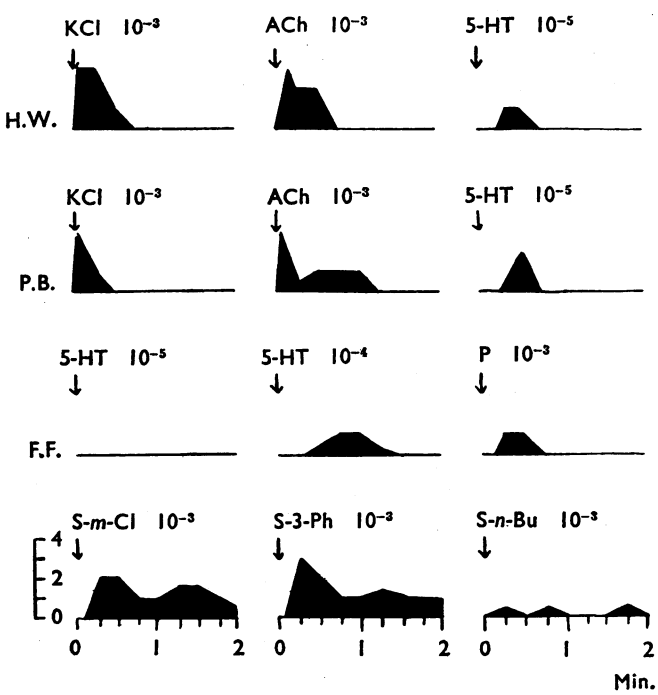


FIG. 4.—Diagrams indicating the intensity of cutaneous pain experienced by 3 observers (H.W., P.B., and F.F.) when certain drug solutions were applied to the exposed base of blisters. Note the distinct delay in the response to 5-hydroxytryptamine (5-HT) and phenyldiguanide (P). The amidine derivatives tested on F.F. after P were *S*-*m*-chlorobenzylisothiourea (S-*m*-Cl), *S*-3-phenylpropylisothiourea (S-3-Ph), and *S*-butylisothiourea (S-*n*-Bu). All concentrations are given in g./ml. KCl, potassium chloride; ACh, acetylcholine. Vertical scale gives the numerals indicating intensity of pain (see text).

effect. Furthermore, the time course of the effect was generally that expected from the previous reaction of the subject, namely an almost immediate response to acetylcholine, histamine, or potassium chloride, but a delayed response to 5-hydroxytryptamine or phenyldiguanide (Fig. 4). However, when some of the drugs were given repeatedly at short intervals, sensitivity to them rapidly diminished. For example, when F. N. F. was given five successive doses of 5-hydroxytryptamine, he experienced considerable pain with the first dose, though the concentration of 5-hydroxytryptamine was only 10^{-8} g./ml., some pain with the second dose, which was 100 times stronger, and subsequently no pain at all with concentrations of 10^{-5}, 10^{-4}, and 10^{-7} g./ml. Yet he remained sensitive to histamine and potassium chloride, though not to tryptamine. Similar results were obtained with the other subjects. Treatment with phenyldiguanide resulted in desensitization to the pain-producing action of 5-hydroxytryptamine. Phenyldiguanide and other amidine derivatives (*S*-benzylisothiourea, *S*-*m*-chlorobenzylisothiourea, *S*-(3-phenylpropyl)isothiourea) resembled 5-hydroxytryptamine in causing delayed pain. A more rapid pain was caused by still other amidine derivatives (guanidine, *asym.*-dimethylguanidine), which do not evoke circulatory and respiratory reflexes in small doses.

Other Effects

Toxicity

Mice that had received 10 mg./kg. of phenyldiguanide intraperitoneally were sedated and showed some respiratory irregularity; breathing was alternately fast and slow. A dose of 20 mg./kg. produced similar effects with hunching of the back. With 50 mg./kg., there was almost immediate respiratory irregularity but less sedation than with the smaller doses. 100 mg./kg. produced similar effects at first; prolonged tachypnoea developed 5 to 10 min. later. When injected intraperitoneally in doses of 230, 250, 280, and 300 mg./kg., phenyldiguanide killed 3, 7, 9, and 10 mice respectively out of batches of 10. Death was usually delayed for an hour or more. It was preceded by utter prostration, laboured breathing, and anoxic convulsions.

Lowering of Rectal Temperature

An intraperitoneal dose of phenyldiguanide (100 mg./kg.) did not lower the rectal temperature of mice to any greater extent than did a control injection of saline. In contrast, 5-hydroxytryptamine lowered rectal temperature considerably even in doses of 10 or 20 mg./kg. When 5-hydroxytryptamine was given by the intracerebral route, a dose of 1 μg. sufficed to lower appreciably the rectal temperature of treated mice, much smaller falls in rectal temperature being obtained with mice that had been given control injections of saline.

Prolongation of Sleeping Time

It has been shown (Fastier, Speden, and Waal, 1957) that 5-hydroxytryptamine prolongs the average sleeping time of mice that have been given a standard dose of chloral hydrate or a barbiturate, probably by lowering body temperature. The results of three experiments with phenyldiguanide are given in Table I. It can be seen that 10 and 50 mg./kg. of phenyldiguanide did not affect sleeping time after chloral hydrate, but 100 mg./kg. produced a highly significant increase.

TABLE I

EFFECT OF PHENYLDIGUANIDE ON CHLORAL HYDRATE SLEEPING TIME

To indicate the broad trend of the results, the geometrical mean for the sleeping times of batches of mice are given in the second column. However, "t" was calculated from log. sleeping times, as described under "Methods."

Dose	Mean Sleeping Time	"t"
10 mg./kg.	21·8 min.	
saline	20·3 ,,	0·40
50 mg./kg.	18·8 ,,	
saline	17·5 ,,	0·28
100 mg./kg.	35·8 ,,	
saline	17·3 ,,	7·2

Actions on Gut

Gastric haemorrhage occurs in mice after large doses of 5-hydroxytryptamine (Blackman, Campion, and Fastier, 1959). Phenyldiguanide or *S*-(3-phenylpropyl)isothiourea (100 mg./kg.) given subcutaneously 6 hr. before the mice were killed and their stomachs examined did not produce such haemorrhages.

Judging by the number and consistency of the faecal pellets after treatment, phenyldiguanide was much less active than 5-hydroxytryptamine in stimulating peristalsis in mice. On the other hand, inspection of the abdominal viscera showed that in rabbits phenyldiguanide, like 5-hydroxytryptamine, caused an increase in peristaltic activity when injected intravenously in small doses (50 to 100 μg./kg.). This effect was less evident in experiments on cats.

The rat fundal strip preparation of Vane (1957), which was contracted by 5-hydroxytryptamine in

doses of as little as 10^{-9} g./ml., was not contracted by phenyldiguanide in concentrations up to 10^{-5} g./ml., nor was the response to 5-hydroxytryptamine affected by the administration of phenyldiguanide.

Action on Striped Muscle

Phenyldiguanide in concentrations of up to 50 μg./ml. did not affect the maximal contractions of the rat diaphragm preparation of Bülbring (1946), stimulated directly or through the phrenic nerve.

Discussion

There is a remarkably close similarity between some of the actions of phenyldiguanide and corresponding actions of 5-hydroxytryptamine. And yet there is no general similarity between the properties of phenyldiguanide and those of 5-hydroxytryptamine.

Points of Difference

Some of the characteristic properties of 5-hydroxytryptamine are largely or entirely lacking in phenyldiguanide. Whereas in our experiments on perfused rat blood vessels 5-hydroxytryptamine constricted blood vessels almost as powerfully as adrenaline, phenyldiguanide did not affect vessel tone. Phenyldiguanide did not raise the tone of rat fundal strips, though these were highly sensitive to 5-hydroxytryptamine. Phenyldiguanide did not produce gastric haemorrhage in the mouse. Moderate doses of phenyldiguanide did not prolong chloral hydrate sleeping time; after a large dose of phenyldiguanide a significant prolongation was seen, but this was not accompanied by a fall of body temperature, such as has been observed in experiments with 5-hydroxytryptamine and which may explain the prolongation by 5-hydroxytryptamine of sleeping time after chloral hydrate (Fastier, Speden, and Waal, 1957). As noted previously (Fastier and Waal, 1957), phenyldiguanide lacks the strong antidiuretic action of 5-hydroxytryptamine.

Points of Similarity

Several types of sensory nerve endings which are stimulated (not necessarily directly) by 5-hydroxytryptamine respond similarly to phenyldiguanide. A resemblance between the two drugs has been noticed in their effects on sensory receptors situated in the heart and lungs (Mott and Paintal, 1953; Dawes and Comroe, 1954; Kottegoda and Mott, 1955; Paintal, 1957), on different types of gastro-intestinal receptor (Paintal, 1954), and on the peristaltic reflex (Bülbring and Lin, 1958). Our experiments in which drugs were applied to the exposed blister base provide evidence for yet another type of sensory receptor which is acted upon by both agents.

The mere fact that amidine derivatives such as phenyldiguanide resemble 5-hydroxytryptamine in producing hypotension, bradycardia and apnoea by reflexes with vagal pathways does not provide sufficient evidence that these compounds are acting at the same sites, for the same triad of effects may be produced by the excitation of various sensory receptors, as emphasized by Dawes and Comroe (1954). Thus, the composite "von Bezold reflex" obtained with typical veratrum alkaloids differs in several important respects from the coronary and allied chemoreflexes elicited by phenyldiguanide. Unlike veratrum alkaloids, phenyldiguanide fails to excite the pulmonary depressor reflex in the dog, though it does so readily in the cat. Again, when transmission through vagal fibres is selectively inhibited by gradual cooling of the vagal trunk, the reflex actions of veratrum alkaloids are blocked long before those of phenyldiguanide (Dawes, Mott and Widdicombe, 1951).

The reflex actions of phenyldiguanide and 5-hydroxytryptamine cannot be differentiated by such means, however. Investigators who have studied both compounds for their reflex actions have been impressed by points of similarity rather than by points of difference. The results of the experiments in which we have compared the reflex depressor effects of 5-hydroxytryptamine and phenyldiguanide under a variety of conditions reinforce the impression that these two drugs are acting at the same sites.

Some Possible Explanations

The very limited pharmacological resemblance of phenyldiguanide to 5-hydroxytryptamine is a strong argument against the possibility that the pharmacological action of phenyldiguanide might be mediated in some way by 5-hydroxytryptamine. Were the effects of phenyldiguanide due to an ability to liberate 5-hydroxytryptamine *in vivo*, then one would expect phenyldiguanide to have the direct as well as the reflex actions of 5-hydroxytryptamine. For the same reason it seems unlikely that phenyldiguanide could act indirectly by potentiating the effects of endogenous 5-hydroxytryptamine. Some of the amidine derivatives which have powerful reflex actions do happen to be powerful inhibitors of amine oxidase (Fastier and Hawkins, 1951). However, the two forms of activity do not run parallel. Further evidence against this idea is

provided by the observation that the reflex effects of phenyldiguanide come on as rapidly as those of 5-hydroxytryptamine and are as evanescent.

Both drugs might act on the same cells but in fundamentally different ways. The gross reactions of a nerve cell are probably few. It is not unlikely that the same end-effects could be produced by drugs which acted on different parts of the same cells or which affected different chemical reactions going on there.

The alternative hypothesis, that phenyldiguanide acts in essentially the same way as 5-hydroxytryptamine at certain sensory nerve endings, implies that the different types of cellular " receptor " for 5-hydroxytryptamine differ so markedly from one another that phenyldiguanide is able to affect only certain of them.

The receptors on which 5-hydroxytryptamine can act may well differ somewhat from one to another as regards accessibility or configuration. Different types of receptor have already been postulated for 5-hydroxytryptamine (Gaddum and Hameed, 1954), as they have been also for adrenaline (Ahlquist, 1948) and yet other agents.

Blocking Agents.—Perhaps the most convincing evidence that phenyldiguanide and 5-hydroxytryptamine act on the same receptors is provided by the experiments with 2-naphthylguanidine and with bufotenine. It did not prove possible to antagonize the reflex depressor action of 5-hydroxytryptamine by giving one of these blocking agents without producing a similar modification of the response to phenyldiguanide. The blocking action of 2-naphthylguanidine is probably exerted on those sensory receptors which are stimulated by phenyldiguanide, since there is chemical similarity between the compounds and since 2-naphthylguanidine acts very much like phenyldiguanide when it is given in smaller doses than those having a blocking action. Similarly, the blocking action of bufotenine is probably exerted on those sensory receptors which are stimulated by 5-hydroxytryptamine. We therefore think it significant that the reflex depressor actions of both phenyldiguanide and 5-hydroxytryptamine can be blocked by giving *either* 2-naphthylguanidine or bufotenine. Presumably, all four compounds have a common site of action, but only 2-naphthylguanidine and bufotenine form sufficiently stable combinations with the receptors to prevent further excitation for a considerable period. Blocking of reflex actions has been observed previously with *S*-nonylisothiourea and with 5-amino-3-ethyl-2-methylindole (Dawes and Comroe, 1954). It seems likely that these effects are closely similar to those described in the present work.

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