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GUANIDINE DERIVATIVES IN MEDICINE

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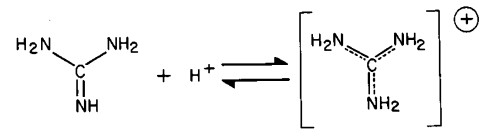
FIFTY years have passed since the introduction of insulin into medicine. It is not widely known that guanidine derivatives were first used in the therapy of diabetes at about the same time, and that the effects of guanidine in a biologic system were first described nearly 100 years ago.[1] Over the years, a wide variety of guanidine derivatives have become important investigational tools, as well as major therapeutic agents. Table 1 shows some of these compounds and the extensive range of their activities and uses. It is tempting to conclude that the common structural feature, the guanidine residue, confers no more specificity on this group of compounds than, for instance, an amino group. Although this conclusion may be partially justified, certain biologic responses to the guanidine derivatives seem to distinguish them as a group from other organic bases. It is also obvious that for each compound, the portion of the molecule other than the guanidine group must confer much of its specificity. This presentation will explore in detail the two major structural features of guanidine derivatives: the positively charged guanidine base itself and the side chain or substituent groups, many of which are quite hydrophobic. Of course, any single molecule with both charged and hydrophobic structures can potentially act as a cationic detergent. Considering their high degree of pharmacologic selectivity and potency, however, it seems unlikely that the agents listed in Table 1 are simply acting as nonspecific detergents. Similarly, the well known protein-denaturant properties of guanidine, observed at concentrations of about 6 M, are almost certainly irrelevant to the pharmacologic actions of the guanidine derivatives.

Table 1. Guanidine Derivatives and Their Uses.

AGENT	USES	
	LABORATORY	CLINICAL
Guanidine	Neuromuscular studies	Botulism
	Inhibition of poliovirus replication	Myasthenia gravis
Guanethidine	Adrenergic blockade	Hypertension
Phenylbiguanide	Reflex depression	–
Phenethylguanidine	Antiarrhythmic	–
Phenethylbiguanide	Mitochondrial inhibition	Diabetes
	Activation of fibrinolysis	Thrombotic diseases
Alkyl monoguanidine	Monoamine oxidase inhibition Mitochondrial inhibition	–
Amiloride	Membrane transport studies Ganglionic blockade	Diuretic
Tetrodotoxin	Inhibition of electrically excitable membranes	–
Chloroguanide pyrimethamine	–	Malaria
Chlorophenyl isopropyl biguanide	–	Malaria
Decamethylenediguanide	Diamine oxidase inhibitor Antitrypanosomal agent	(Diabetes)

STRUCTURE AND BIOLOGIC PROPERTIES OF THE GUANIDINE RESIDUE

Guanidine is a strong organic base with a pK_a of about 12.5. At physiologic pH, therefore, all but a small fraction of the guanidine molecules in solution exist as the positively charged species; that small fraction of uncharged molecules cannot be ignored, however, since it may have an important role in the pharmacology of the guanidine derivatives (Fig. 1).



GUANIDINE

Figure 1. Chemical Structure of Guanidine.

A second chemical feature of guanidine is the symmetry of the protonated molecule, which provides stability. N-substitution destroys the symmetry, and it is, in part, the tendency to resume the symmetrical, unsubstituted form that renders the phosphate-substituted guanidine derivative, creatine phosphate, a high-energy compound.[2]

Other important features of guanidine are its size and shape: a planar molecule with bond distances of $A_{13.2\ nm}$ between the central carbon and each nitrogen.[3] As first pointed out to me by Dr. Howard Frazier, this probably makes the guanidinium molecule considerably smaller than either the hydrated sodium or potassium ion. Guanidino groups in proteins apparently form tighter complexes with anions than do the corresponding ε-amino groups, evidence that the size and shape of the guanidino moiety affect charge distribution within the molecule.[4]

Of course, the amino acid arginine contains a guanidino group, and, as already noted, so do creatine and

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creatinine. Guanidine itself and other derivatives have repeatedly been sought as natural constituents of biologic fluids.[5] Despite considerable effort, however, a role for metabolites such as methylguanidine and dimethylguanidine or guanidine itself, even in pathologic states such as uremia, has not been convincingly demonstrated.[6] Guanidinosuccinic acid has recently been suspected of contributing to the hemostatic difficulties in uremia, but its ultimate importance has yet to be determined.

The first clues to the biologic specificity of guanidine were apparent in the earliest studies of this compound in 1876.[1] Guanidine injected into frogs produced tonic extensor spasms, as well as peripheral muscle twitchings that were abolished by curare. Some 30 years later, the similarity of these effects to those of univalent alkaline metals on neuromuscular excitability was pointed out by Führer,[7] who further noted that the action of both guanidine and the alkali metal ions was antagonized by calcium. It was this observation that, through a remarkable series of mistaken interpretations, led ultimately to the development of the antidiabetic biguanides.[8] More important for our discussion was the introduction of the idea that guanidine and its derivatives could interact specifically with biologic metal-ion receptor sites. In the 1950's, guanidinium was found to be capable of substituting completely for sodium in the extracellular fluid of frog axons to maintain electric excitability[9]; it was therefore inferred that guanidinium was specifically sodium-like. Since then, many other investigators have explored the interaction of guanidine and guanidine derivatives of widely differing structure with sodium-dependent systems. The most important of these studies are shown in Table 2.

It is apparent from this table, however, that only in the nerve axon was guanidine actually able to replace sodium; in all the other studies, the guanidine derivative blocked or altered a sodium effect, which could have resulted from actions at sites other than the sodium-specific sites. More recently, even the ability of guanidine itself to replace sodium in maintaining axon excitability has lost its implication of specificity, since a variety of highly polar, polyatomic univalent organic cations other than guanidinium have been shown to be capable of replacing sodium.[10]

Table 2. Studies Demonstrating Interaction between Guanidinium Derivatives and Sodium in Biologic Systems.

Guanidinium Compound	Biologic System	Nature of Response
Guanidine	Squid axon; frog nerve.	Substitutes for sodium in maintaining action potential[9,10]
Guanidine	Toad bladder	Competes kinetically for sodium transport[11]
Amiloride	Toad bladder	Noncompetitively inhibits sodium transport[12]
Tetrodotoxin	Nerve; muscle membrane.	Blocks sodium movement[13]
Octylguanidine	Mitochondria	Competes with sodium activity on mitochondrial function[14]
Guanethidine	Toad bladder	Stimulates sodium transport[15]

Evidence for the absolute biologic specificity of the guanidinium residue has been weakened by another observation: several classes of positively charged quaternary ammonium compounds have been shown to have pharmacologic behavior closely resembling that of guanidinium compounds (Fig. 2). The hypoglycemic properties of phenethylbiguanide and the isoxazolyl derivative of the quaternary ammonium compound, methyl pyridinium, appear to be very similar,[16] although these data are preliminary. More convincing evidence is derived from studies with antihypertensive agents. The prototype compounds guanethidine and bretylium, which contain guanidine and quaternary ammonium residues respectively, affect catecholamine release from adrenergic-nerve terminals by demonstrably different mechanisms.[17] Although the compounds containing the guanidinium group separated from a ring structure by two methylene carbons all exerted guanethidine-like properties, their activity could be changed to that resembling bretylium simply by reduction of the separation between ring and basic group from two methylene carbons to one (Fig. 2).[18]

Influence of Substituent Groups

These observations with bretylium-like and guanethidine-like adrenergic blockers imply that the structural requirements for activity at the positively charged end of the molecule are not absolute. At the same time, these studies emphasize the importance of the uncharged, hydrophobic portion of the guanidine derivative.

During the studies of nerve axon, addition of an alkyl group to guanidinium was found to eliminate

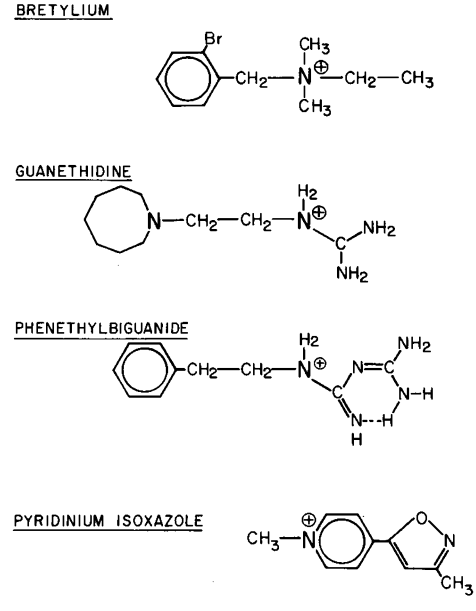

Figure 2. Guanidine-Derived and Quaternary Ammonium Agents with Related Activities.

the ability to sustain electric excitability in the absence of sodium.[9] Furthermore, alkyl-substituted guanidines such as n-amyl guanidine manifested an opposite effect; these compounds actually rendered the nerve fibers inexcitable more rapidly than simple removal of sodium, and markedly delayed and reduced the restoring activity of guanidinium and sodium.[9] Arginine, with the same number of carbons in the chain as the n-amyl derivative, but with a highly polar carboxyl group at the end opposite the guanidinium, was quite inert so far as effects on electric activity were concerned.

Studies of antidiabetic guanidine derivatives provide another example of the importance of the uncharged end of the molecule. After the observation that guanidine produced hypoglycemia in animals,[8] exploration of the hypoglycemic activity of the naturally occurring alkaloid, 4-amino-n-butyl-guanidine, gave the first clue that the hypoglycemic potency of monoguanidine derivatives could be increased by the addition of a hydrophobic side chain.[19] An immense amount of work has since been devoted to analysis of the structure-function relations of hypoglycemic guanidine derivatives. Out of these studies emerged the first clinically useful hypoglycemic drug, decamethylene diguanide, or Synthalin A. This strongly hydrophobic compound was apparently produced by accident during attempts to make a long-chain homologue of agmatine,[19] and it was soon discovered that elongation of the methylene chain from 10 to 12 carbons produced the even more potent Synthalin B.

The immense success of insulin, coupled with the hepatic and renal toxicity of the Synthalins, eclipsed the clinical use of guanidine derivatives in diabetes, and they were ignored for 30 years. Thus, even though the group of compounds termed biguanides* had been studied along with many other guanidine derivatives in the 1920's,[20] it was not until further studies were done in the late 1950's that their relatively lower toxicity and good hypoglycemic activity became apparent. Shapiro and his colleagues examined biguanides with a great array of hydrophobic side chains, and among their most revealing findings was the varying hypoglycemic potency of the n-alkyl biguanide compounds. This series of compounds, the structures of which differed only by the number of carbons in the straight-chain substituent, showed moderate hypoglycemic activity at short-chain lengths, for example, C_{1-3}; activity rose to a maximum at C_{5-6}, and then fell off to 0 at C_{10} and above.[21] The immense importance of side-chain structure was again evident here, although the variations in structure were not quite as critical for activity as in the adrenergic blockers. These changing structure-function relations within an n-alkyl series of drugs are certainly not unique to biguanides; similar data for cardiovascular effects of n-alkyl thiouronium compounds have been described in detail.[22]

Enlargement of the charged, basic end of the molecule from monoguanide to biguanide also appeared to have enhanced the potency and selectivity of the hypoglycemic response, although, as noted above, certain monoguanidine derivatives were also effective in lowering blood sugar. Many monoguanidines, on the other hand, were also potent adrenergic blockers. The overlap of these two activities in a single molecule was perhaps best expressed in phenethylguanidine, which in careful studies in cats and mice exhibited guanethidine-like adrenergic blocking activity,[23] whereas in the rabbit it was a potent hypoglycemic agent.[24] Of particular interest in these rabbit studies was an initial hyperglycemic response to injection of phenethylguanidine; the hyperglycemia could be eliminated by ergotamine or surgical adrenergic blockade, thereby unmasking early hypoglycemic effects. These findings indicated that, just as with parenteral guanethidine, catecholamines were initially released into the circulation in response to phenethylguanidine. Therefore, the recent report that the antihypertensive agent guanethidine improved glucose tolerance in patients with maturity-onset diabetes[25] came as no surprise.

Mechanisms of Hypoglycemia from Guanidine Derivatives

The exact mechanism by which guanidine and guanidine derivatives cause hypoglycemia has been a subject of great interest for many years. Soon after their introduction, several features of guanidine pharmacology became apparent: the metabolic effects of Synthalin and of insulin (on hepatic glycogen, for example) were not identical[26]; oxygen consumption was dramatically suppressed by Synthalin[26]; and guanidine hypoglycemia was accompanied by severe lactic acidosis.[27] Very early, then, inhibition of cellular respiration, with consequent acceleration of glycolysis by the Pasteur effect, was implicated as the basis of hypoglycemia. Later studies with the biguanides directly demonstrated inhibitory effects in isolated tissues such as muscle and heart[28]; the observation that guanidine, alkylmonoguanidines and biguanides were all effective inhibitors of respiration in isolated mitochondria[29] was generally taken as further supporting evidence. Finally, the inhibition of gluconeogenesis by biguanides in intact animals[30] and in isolated perfused liver,[31] presumably through interference with oxidative energy production, also suggested that hypoglycemia results from a general poisoning of energy metabolism.

Unfortunately for this simple hypothesis, but fortunately for diabetic patients, the data do not all fit. First of all, most of the guanidine-derivative effects in animals and isolated tissues just described could be shown only with doses or levels at least 20 and often several hundred times greater than the usual therapeutic levels in human patients. Secondly, the longer-chain alkylbiguanides, which, as noted, have no hypoglycemic activity,[21] are even more potent respiratory inhibitors in

*Diguanide is the correct chemical nomenclature for compounds containing two separate guanidinium residues within a single molecule, whereas biguanide refers to a structure in which two monoguanides share a common nitrogen.

vitro than the shorter-chain hypoglycemic compounds[32]; the consequences of the respiratory inhibition appear to be identical for hypoglycemic and nonhypoglycemic members of the n-alkyl biguanide series.[33] Thirdly, the elevations in blood lactate associated with the usual therapeutic doses of biguanides in human subjects are extremely small[34] and not entirely consistent. Finally, at least in normal human subjects, there is good evidence from glucose-kinetic studies that phenethylbiguanide in moderate doses does not inhibit respiration or gluconeogenesis.[35] In fact, in the presence of the drug, glucose is converted to lactate by peripheral tissues more rapidly than under normal circumstances; that lactate is then recycled through the liver back to glucose at an accelerated rate. In normal subjects the rates of glycolysis and gluconeogenesis are exactly balanced so that under the usual overnight fasting conditions the blood sugar does not fall.

These considerations point to two qualitatively different effects of guanidine derivatives on carbohydrate metabolism. First of all, high doses, studied mostly in laboratory animals, produce respiratory inhibition, acceleration of glycolysis, breakdown and depletion of tissue glycogen and inhibition of gluconeogenesis with resultant hypoglycemia and lactic acidosis. It is a reasonable assumption that the respiratory inhibition is causally related to the other effects. Secondly, at low doses, a much more subtle effect occurs, which until recently had been observed only in normal human subjects: peripheral glycolysis is accelerated without alterations in mitochondrial energy-producing reactions, hepatic gluconeogenesis is not directly affected, and glycogen stores are increased. Recently, the effects of high-dose and low-dose therapy with biguanides have been carefully and systematically reinvestigated in animals.[36] Not only was the essential difference confirmed between high-dose and low-dose effects on glucose conversion to carbon dioxide, on glycogen stores and on gluconeogenesis, but another very important observation emerged. Whereas high-dose biguanide hypoglycemia could be achieved in both normal and alloxan-diabetic animals without any insulin supplementation, low doses produced their accelerating action on glucose disposition only in normal animals or in diabetic animals that had received insulin. This requirement for the presence of insulin suggested that low-dose biguanide effects may be achieved through "amplification" of the peripheral action of insulin.

Importance of Nonionic Diffusion for the Pharmacology of Guanidine Derivatives

For high doses of guanidine derivatives to inhibit mitochondrial activity in vivo, it appears that the drug must cross the plasma membrane and enter the cell interior in relatively large quantities. It is well known, however, that small organic bases such as ammonia, as well as larger ones such as quinidine, distribute across membranes mainly by the process of nonionic diffusion. They are trapped in the ionic form in the more acidic compartment. The distribution of biguanides appears to reflect the acid-base relations among the body compartments; in particular, large amounts accumulate in the lumen of the stomach[37] — a finding consistent with a mechanism of nonionic diffusion. The same mechanism can explain the initial uptake against a concentration gradient that we have demonstrated in mitochondria,[38] and may account for the marked potentiation of biguanide inhibition of gluconeogenesis in the isolated perfused liver when the extracellular perfusate pH is increased.[39]

Occasional patients receiving biguanide therapy, particularly those with some impairment of renal function,[40,41] have been suspected of accumulating excess amounts of these drugs in the intracellular compartment, with consequent high-dose effects on tissue respiration and resultant hypoglycemia and lactic acidosis. If biguanides are, in fact, distributed across membranes by nonionic diffusion, alkalinization of the extracellular fluid during bicarbonate therapy might make the situation worse by driving more of the drug into cells. Similarly, alkalinization of the urine would be expected to decrease renal excretion of the drug. However, we need to know considerably more about the possible relation between biguanide therapy and lactic acidosis, as well as the transport, distribution and excretion of biguanides, before we can make informed decisions concerning this potentially serious condition.

Possible Molecular Mechanisms of Low-Dose Biguanide Hypoglycemia

The indication that biguanides in low concentratrations augment or amplify insulin activity not only has important therapeutic implications, but also raises intriguing questions about the mechanism of this amplifier effect and of the action of insulin itself. It may be useful to explore a hypothesis that, although not new,[22] has only recently been tested directly and may clarify the relation between the activity of biguanides and of insulin. Briefly stated, biguanides bind to divalent metal-ion sites in membranes; through displacement of divalent metals, particularly calcium, the characteristics of the membrane are changed in a manner that augments the effects of insulin.

The evidence to support this hypothesis now seems considerable. Some evidence that guanidine might displace calcium from important tissue sites has existed since the first neuromuscular studies by Führer 70 years ago.[7] Recent investigations using more refined technics have confirmed the similarity of the effects of guanidine and calcium depletion at the neuromuscular junction.[42] This relation was strengthened by the demonstration by Minot and her colleagues that calcium alone could prevent the shock, lactic acidosis and hypoglycemia of guanidine intoxication.[27] A calcium-guanidine derivative interaction has also emerged from studies with the guanidinium-containing compound, tetrodotoxin. Although this potent inhibitor of nerve and muscle excitability is generally considered a specific inhibitor of transmembrane sodium movement, tetrodotoxin has also been shown to block axo-

nal activity in a medium containing only calcium.[43] Alkylguanidines, although without effect on mitochondrial dinitrophenol-stimulated ATPase, are highly effective in blocking the calcium-induced ATPase.[29]

In an effort to define the metal ion-guanidinium interaction further, we have recently examined the effects of a variety of guanidine derivatives in a model system using purified pyruvate kinase.[44] This enzyme has highly specific ion requirements and is dependent for activity on divalent transition metals such as manganese. In this system, phenethylbiguanide was a relatively potent inhibitor: the most interesting result, however, was that even though biguanides in solution are univalent cations, they inhibited by displacing activating divalent metal ions from enzyme-binding sites[42]; the nonactivating divalent cation, calcium, was also displaced. In a series of structurally related compounds, the biguanides were more effective inhibitors than the corresponding monoguanidines, which in turn were more effective than the amines, but all interacted with the divalent metal ion-binding site. Overall, however, the hydrophobic portion of the molecule was more important for the binding of phenethylbiguanide to the enzyme than the ionic portion. In a general way, then, these structure-inhibitor correlations paralleled the effects of structural changes on hypoglycemic activity.

How might the displacement of divalent metal ions from plasma-membrane binding sites by guanidine derivatives increase the apparent effectiveness of insulin? Insulin binding to membrane sites could be enhanced by the presence of the guanidine compound, but there are no direct data on this point. A second possibility is that once bound, insulin might exert some of its effects by displacing calcium from the membrane. This hypothesis has now received direct experimental support: insulin has been shown to displace $^{45}Ca^{++}$ from plasma-membrane vesicles of rat liver, whereas catecholamines and glucagon increased the quantity of bound calcium.[45] The cascade of reactions resulting from the presentation of insulin to a responsive tissue might be as follows: insulin binding to specific plasma-membrane binding sites; alteration of membrane configuration; displacement of bound calcium from adjacent membrane sites; and alteration in glucose permeability, sodium and potassium fluxes and adenyl cyclase. In this scheme, biguanides and, possibly, the pyridinium derivatives could enhance certain effects of insulin by increasing the displacement of bound calcium. Although the precise coupling between displacement of membrane-bound calcium and increase in glucose permeability is not immediately evident, some precedent for such a coupling mechanism may exist, since it now appears that the well known increase in glucose permeability in the plasmalemma of skeletal muscle observed during exercise is probably mediated by an increase in intracellular calcium concentration.[46] In any event, this hypothesis is testable and, if confirmed, may contribute to our understanding of the fundamental actions of the guanidine derivatives.

Discussion

Dr. Martin Nothmann: Before the studies of the biguanides were done on diabetes, I wrote a paper about tetany as a manifestation of guanidine toxicosis. That will interest you particularly in your studies about calcium and guanidine. Later on, there was a physiologist in Hamburg who showed that in tetany the concentration of guanidine is increased in the blood; it is also increased in hyperventilation tetany. The second point that I want to make is that if one is very careful in giving small enough doses of Synthalin, one can demonstrate an increase in glycogen.

Dr. Davidoff: Yes; the latter effect has been observed by a number of investigators: with small doses of butylbiguanide, glycogen deposition is increased and cell respiration does not seem to be poisoned.

Dr. Richard Field: Would you review the information that indicates that insulin must be involved with a low-dose effect of biguanide?

Dr. Davidoff: This information is based on tracer studies, primarily with labeled glucose. $^{14}CO_2$ production from an injected dose of labeled glucose in these animals is not altered in diabetic animals when biguanide alone is injected, but biguanides increase $^{14}CO_2$ production from glucose when the animals have also been given a small amount of insulin. Biguanide in this setting also increases the arabinose-distribution space in an experiment analogous to the classic Levine study with galactose. Biguanides also affect isolated tissues from diabetic animals. When a small amount of insulin is included in the medium, low concentrations of biguanide increase glucose uptake and glycogen deposition, whereas in the absence of insulin, the biguanide has no effect. When insulin is present in supramaximal amounts, one cannot demonstrate that low-dose biguanide has any further effect on the tissue.

Dr. George Cahill: Do you think inhibition of glucose transport in the gut is possibly related to altered sodium transport?

Dr. Davidoff: That is a good question. Biguanides in the usual therapeutic doses inhibit gut glucose transport, an effect that may contribute to the improvement of oral glucose tolerance in diabetic patients. The mechanism of that change is not known. Sodium and, possibly, calcium appear to be involved in the uphill movement of glucose into the gut mucosa. Biguanides also inhibit sodium-linked amino-acid transport across the gut mucosa.

Dr. Stuart Schlossman: Is it known whether biguanides, in exerting their usual hypoglycemic effect, actually get into cells?

Dr. Davidoff: It appears that the high-dose effects are accompanied by mitochondrial respiratory inhibition, so it seems very likely that under these circumstances the biguanides are inside the cell; I do not know of any direct evidence on this point. The suggestion that biguanides exert their low-dose action at the cell membrane is inferential.

Dr. Edgar Henshaw: Is the effect of biguanides dif-

ferent from that of simple removal — with a chelating agent, for example — of the calcium from a membrane or enzyme?

DR. DAVIDOFF: Biguanides chelate certain divalent metal ions such as cobalt and nickel. In the studies with pyruvate kinase, we were very concerned about this problem. It turns out that there is no evidence that the biguanides chelate either calcium or magnesium; they therefore are probably not exerting these divalent metal-ion-displacing effects simply by pulling the metal ions off. Furthermore, phenethylguanidine, the monoguanide derivative, is almost as good an inhibitor of pyruvate kinase as the biguanide, and its kinetic effects on the displacement of divalent metal ions are qualitatively identical to the biguanide. Since the monoguanidines do not chelate divalent metals, I think that it all adds up not to a chelator-type phenomenon, but rather to a direct competitive one. Finally, we have directly measured binding of phenethylbiguanide to the enzyme — it binds to the enzyme in amounts consistent with the direct-displacement hypothesis.

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