The Mechanism of Action of Hypoglycemic Guanidine Derivatives

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Renewed interest in guanidine derivatives as possible oral therapeutic agents in the treatment of diabetes has followed the discovery by Ungar et al.[1] that certain biguanides are hypoglycemic agents of low toxicity. This report is concerned with the mechanism of action of this new group of compounds as exemplified by one of them, phenethylbiguanide (DBI), as compared with that of other guanidine derivatives. As long ago as 1918, Watanabe[2] showed that guanidine elicited a pronounced hypoglycemic response in rabbits. However, the toxic manifestations of this substance precluded its trial as a possible therapeutic agent in diabetes mellitus. An intensive search by Frank et al.[3] led to the synthesis of decamethylenediguanidine (Synthalin) which was found to exhibit enhanced hypoglycemic activity associated with markedly diminished toxicity. However, after intensive clinical trial the therapeutic use of Synthalin as an oral hypoglycemic agent was abandoned. Disagreeable side effects including nausea and weakness were frequent. Furthermore, except for lowering blood sugar and diminishing glycosuria, Synthalin therapy did not really correct the aberrant metabolism of the diabetic individual. Elevations in blood and urinary lactate, citrate and other organic acids were observed.[4] In a balance study of diabetics undergoing Synthalin therapy, Kaufmann-Cosla and Vasilco found that urinary glucose was supplanted by large excretions of other organic compounds.[5]

In vitro studies on the effect of phenethylbiguanide (DBI) on rat diaphragm by Williams et al.[6] have demonstrated increased glucose uptake, increased lactate production and increased glycogenolysis. These results are indicative of the action of a respiratory inhibitor. Randle and Smith[7] have demonstrated increased glucose uptake by isolated rat diaphragm subjected to oxygen lack as well as a number of cytotoxic substances including dinitrophenol, cyanide and arsenite. Steiner and Williams[8] have studied the effect of Synthalin and phenethylbiguanide on subcellular preparations from various tissues and conclude that a major site of inhibition of cellular respiration involves cytochrome oxidase. Wick and Larsen,[9] on the other hand, report that cytochrome oxidase is not inhibited, but rather some point prior to cytochrome c in the succinic oxidase system. These authors, working with rat epididymal adipose tissue, also observed that the degree of inhibition of oxidation of several Krebs cycle intermediates by phenethylbiguanide differed considerably: 48 per cent for succinate, 16 per cent for fumarate and only 3 per cent for citrate. In our own experience with rat kidney homogenates we observed a 58 per cent inhibition of fumarate oxidation and a 49 per cent inhibition of succinate oxidation with 2×10^{-3} M phenethylbiguanide.

Hollunger[10] has recently advanced another view concerning the nature of the inhibition of oxygen uptake by guanidine and its derivatives. Working with rabbit kidney mitochondria and rat liver mitochondria, he observed that when phosphorylation became uncoupled from oxidation either by aging, treatment with calcium ions, or by the addition of an uncoupling agent such as 2,4-dinitrophenol (DNP), guanidine no longer inhibited oxygen uptake. Partial restoration of the phosphorylative capacity of the aged or calcium treated mitochondria by addition of diphosphopyridine nucleotide (DPN), adenosine triphosphate (ATP) and manganous ions was accompanied by partial return of susceptibility to guanidine inhibition. Figure 1 illustrates the mechanism postulated by Hollunger to explain these phenomena. Normally, electron transport via the pyridine nucleotide-flavoprotein-cytochrome chain is coupled to the generation of high-energy bonds which eventually

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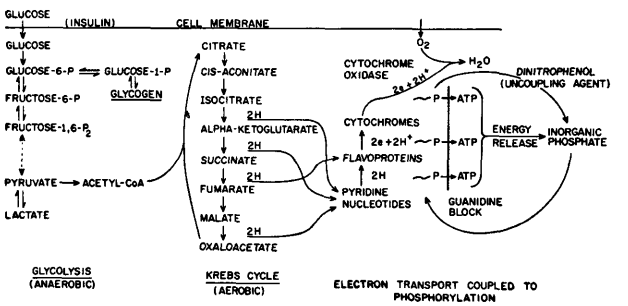

FIG. 1. Scheme showing postulated site of action of guanidine derivatives.

interact with adenosine diphosphate (ADP) to form ATP. These primary high energy bonds must be removed before the respiratory chain can resume electron transport; any block to their removal, in effect, blocks respiration. According to Hollunger, guanidine and its derivatives interfere with the interaction of ADP with the high-energy bond containing chain. Uncoupling agents such as DNP bypass this block by causing wasteful release of respiratory chain high-energy bonds, and, indirectly, release the inhibition of electron transport. Aged or calcium treated mitochondria either do not couple at all during oxidation, or the high-energy bonds formed are unprotected from spontaneous hydrolytic processes; in either case guanidine would not be expected to inhibit oxygen uptake.

To test this hypothesis as a possible mechanism of action of phenethylbiguanide inhibition of oxygen uptake, rat kidney mitochondria were prepared from isotonic sucrose homogenates by differential centrifugation at 0° C., and after several repeated washings and centrifugations, dispersed in a known volume of isotonic sucrose. Figure 2 shows the results of a typical manometric run employing fumarate as a substrate. Each point represents the average ten-minute oxygen uptake of two simultaneously run experiments with the same mitochondrial preparation. Initially there is little difference between the uptake in the control flasks and those with added DNP. However, with time the respiratory rate of the latter gradually declines. The flasks with phenethylbiguanide as the sole addition exhibit a distinctly lower uptake than the controls from the start. Those with phenethylbiguanide plus DNP both added at the beginning show a distinct recovery of respiratory activity during the initial period, but this gradually falls off as do those with DNP alone. The remaining curve shows the effect of DNP addition after twenty minutes upon a phenethylbiguanide inhibited system. There is marked rise in oxygen uptake during the next two ten-minute periods, followed by a gradual decline, generally following the system with DNP alone. Figure 3 shows the same data in terms of accumulated oxygen uptake during the same period of time.

These results are consistent with the mechanism proposed by Hollunger, and are difficult to explain in terms of cytochrome oxidase or succinic oxidase inhibition per se. It is postulated that the in vitro metabolic effects of phenethylbiguanide can be best explained by a primary interference with the transfer of respiratory chain high-energy bonds to ADP, secondarily blocking electron transport, which is reflected in diminution of oxygen uptake. This results in the creation of a partially anaerobic state which diminishes the Pasteur effect which normally limits glycolysis in respiring cells. Glucose uptake and conversion to lactate are therefore increased. At the same time since oxidation is reduced, certain Krebs cycle intermediates such as citrate might be expected to accumulate. This mechanism is consistent with the reported metabolic actions of phenethylbiguanide in humans, although the possibility of differential tissue localization may complicate the over-all in vivo metabolic response.

SUMMARY

1. Phenethylbiguanide inhibits oxygen uptake.
2. Dinitrophenol releases this inhibition.
3. These results are consistent with Hollunger's concept that guanidines inhibit transfer of high-energy bonds to ADP, thereby secondarily inhibiting oxygen uptake.
4. The metabolic effects of phenethylbiguanide, i.e., hypoglycemia, diminished tissue glycogen, and accumulation of citrate, pyruvate and lactate can be interpreted by this mechanism.

SUMMARIO IN INTERLINGUA

Le Mechanismo del Effecto Hypoglycemic de Derivatos de Guanidina

1. Phenethylbiguanida inhibi le acceptation de oxygeno.
2. Dinitrophenol relaxa iste inhibition.
3. Iste resultatos es compatibile con le conception de Hollunger que derivatos guanidinic inhibi le transferimento de ligamines de alte energia a adenosinediphosphato, inhibiente assi secundarimente le acceptation de oxygeno.
4. Iste mechanismo es capace a interpretar le effectos metabolic de phenethylbiguanida, i.e. le hypoglycemia causate per illo, le reducite glycogeno tissutal, e le accumulation de citrato, pyruvato, e lactato.

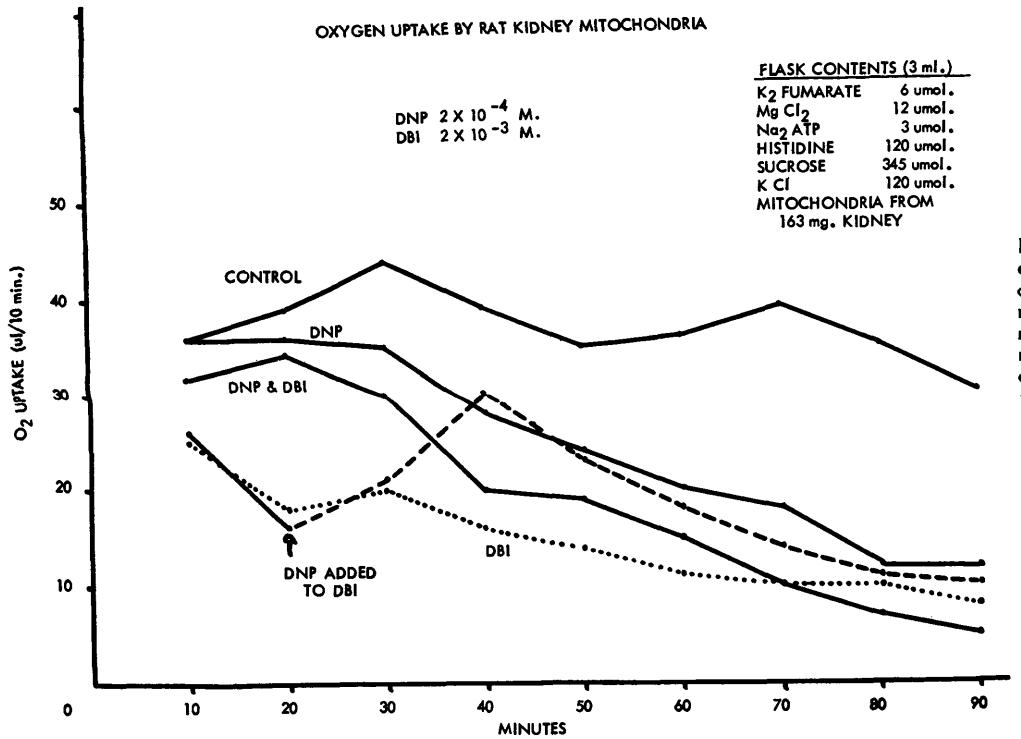

FIG. 2. Effect of phenethylbiguanide (DBI) and dinitrophenol (DNP) on respiration of rat kidney mitochondria utilizing fumarate as substrate. Successive ten-minute increments of oxygen shown on ordinate.

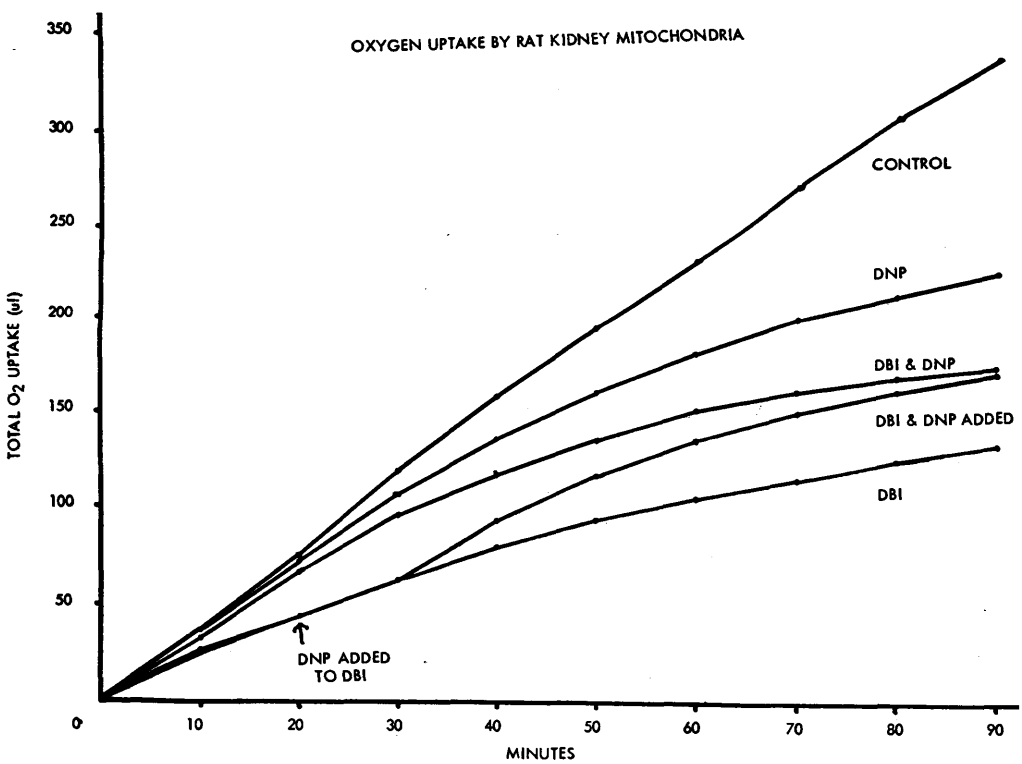

FIG. 3. Same system as in figure 2. Total oxygen uptake shown on ordinate.

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That unesterified fatty acids appear very rapidly in the blood during chylomicron metabolism had been shown by R. J. Havel and D. S. Fredrickson (*J. Clin. Invest.* 35:1025, 1926). They used isotopically labeled chylomicrons which were secured from dogs in which a cannula had been placed in the thoracic lymph duct. Each dog was given 3.6 gm. to 10.8 gm. of fat in the form of cream by stomach tube. The cream contained sodium palmitate-1-C^{14}. The chylomicrons were separated from the lymph, washed, and resuspended in 0.5 M sodium chloride solution. The resulting emulsion was given by intravenous injection to two adult female dogs. These dogs had been fasted for twenty hours prior to the injection. During the injection, the dogs were maintained under light ether anesthesia. Physiologic saline was given by vein during the infusion of the chylomicrons and the collection of subsequent blood samples.

The chylomicrons disappeared exponentially from the blood with a half life of fifteen to twenty-four minutes. The triglyceride and phospholipid fractions of the chylomicrons disappeared at the same rate. The specific activity of the plasma unesterified fatty acids rose during the clearing of the plasma and reached a level exceeding 50 per cent of the specific activity of the transfused triglyceride fatty acids. The work of Havel and Fredrickson showed that the half life of the esterified fatty acid fraction was about two minutes.

Havel and Fredrickson suggested that there is an intimate relationship between the removal of chylomicrons and the hydrolysis of their constituent triglycerides. The site where this hydrolysis of the triglycerides occurs is unknown. Regardless of the tissue responsible for the removal of the chylomicrons from the blood, part of the unesterified fatty acids released during this process is returned rapidly to the blood stream. Here the fatty acids combine with serum albumin and are transported as a protein-fatty acid complex.

The above work indicates that although the unesterified fatty acids form a small fraction of the total blood lipids, their rate of turnover suggests that they play a very important role, especially in the transport of lipids from the adipose tissue to such organs as the liver. The liver appears able to remove these fatty acids from the blood. The rate of removal of the unesterified fatty acids by the liver appears to occur even when no other lipid fractions are being removed from the blood.

With the increasing activity in this field of lipid metabolism, it is likely that additional evidence will soon be forthcoming to delineate more clearly the exact role of unesterified fatty acids in metabolic reactions.

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