Antidiabetic Behavior of Biguanides

F. VICENTE-PEDRÓS ˣ, J. TREJUEQUE MONGE, and F. TOMÁS VERT

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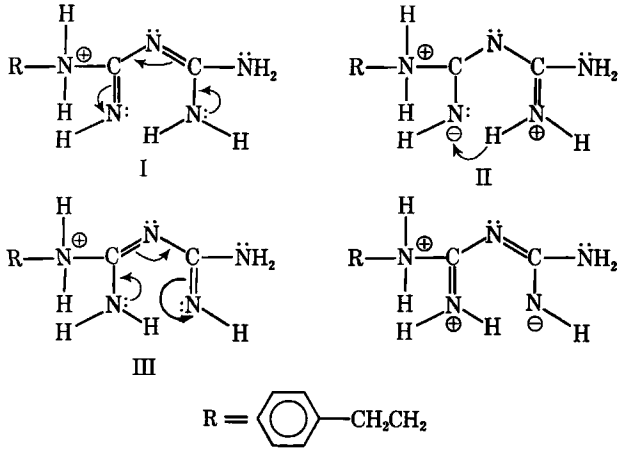
Abstract □ The existence of active electron pairs on some nitrogen atoms in phenformin hydrochloride is inferred from the presence of a hydrogen catalytic polarographic wave. This finding emphasizes the ability of biguanides to form hydrogen bridges with other molecular species such as amino acids and proteins, as well as to form coordination complexes with zinc and other metallic cations by means of these electron pairs. The antidiabetic action of phenformin and other related biguanides can be explained in terms of competition between these molecules and insulin to coordinate cationic oligoelements together with their ability to form hydrogen bonds between the biguanide moiety and insulin itself.

Keyphrases □ Insulin—complexation with zinc *in vivo*, effect of phenformin determination by polarography □ Phenformin—effect on insulin-zinc complexes, determination by polarography □ Polarography—determination of effect of phenformin on insulin-zinc complexes.

Some aspects of the biological behavior of biguanides such as the blood sugar-lowering effect and metabolic interactions have been widely studied (1, 2). Biguanides form stable coordination complexes with divalent cations of oligoelements existing in living organisms. Phenformin [*N*-(2-phenylethyl)imidodicarbonimidic diamide] and other related oral antidiabetic biguanides form very strong complexes with Zn^{2+} and other divalent cations (3). This finding suggests that biguanides produce alterations in the distribution and ratios of divalent cations in biological media (4). It was shown (5, 6) that phenformin administered *in vivo* produced consistent and significant effects on hepatic mitochondrial divalent metal ion content through a more complex mechanism than competitive binding. All of these facts point to the use of biguanides as regulators of oligoelement proportions in living organisms.

Structures for this type of complex have been suggested, but the one reported by Rây and Saha (7) (I) is the most widely accepted.

The antidiabetic action of biguanides (phenformin as an example) can be derived from this complexation phenomenon. This action must be consistent with the following sequence of events:



I II III

R = —CH_2CH_2—

1. Natural insulin is stored in the β-cells of the pancreas in the form of insoluble insulin–zinc granules (8).

2. Biguanides form stronger complexes than insulin does with Zn^{2+}. Formation of such complexes releases insulin from the granules allowing it to go through cellular membranes and enter the systemic circulation.

This scheme is consistent with the physicochemical properties of biguanides and phenformin in particular:

1. These molecules are soluble in biological hydroorganic media, which explains their widely observed, fast conveyance and action (9).

2. At pH values close to human physiological pH, the stable form of biguanides is the cationic monoprotonated form, BH^+ (10). These cations retain the delocalized π-electronic structure of the biguanide group of the free base, as can be seen from the persistence of the 232-nm band of the UV spectra of bases, at pH values less than the pK_{a_2} of biguanides, and in any case at pH values <7. According to a previous report (11), the protonated biguanide group can be formulated in several resonant forms (Fig. 1), all of them with nitrogen atoms exhibiting localized electron pairs, which accounts for the ability of monoprotonated biguanides to form hydrogen bonds and coordination compounds with metallic cations.

These structural features call for the solubilization of the insulin–zinc granules in the pancreatic β-cells, which involve the accessibility of insulin–zinc complexes to biguanides at a molecular level. Such accessibility requires the breaking of the hydrogen bonds that link insulin polyamino acid chains in the building of granules. This breaking process should be enhanced, because of the strong ability of biguanides themselves to form hydrogen bonds. Both the ability to form hydrogen bonds and to chelate with zinc are located at the electron pairs of nitrogen.

This work was directed to characterize this structural feature by using direct current (DC) and superimposed alternating current of first harmonic (AC_1) polarographic techniques on phenformin as a representative of oral antidiabetic biguanides.

EXPERIMENTAL

A polarograph[1] was used and pH measurements were carried out by means of a pH meter[2]. Polarographic parameters were fixed (unless their

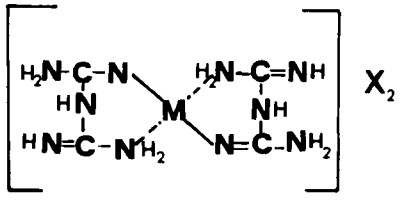


Figure 1—*Resonant forms for biguanide monoprotonated group.*

[1] Metrohm E-506 Polarograph.
[2] Radiometer pH M-62.

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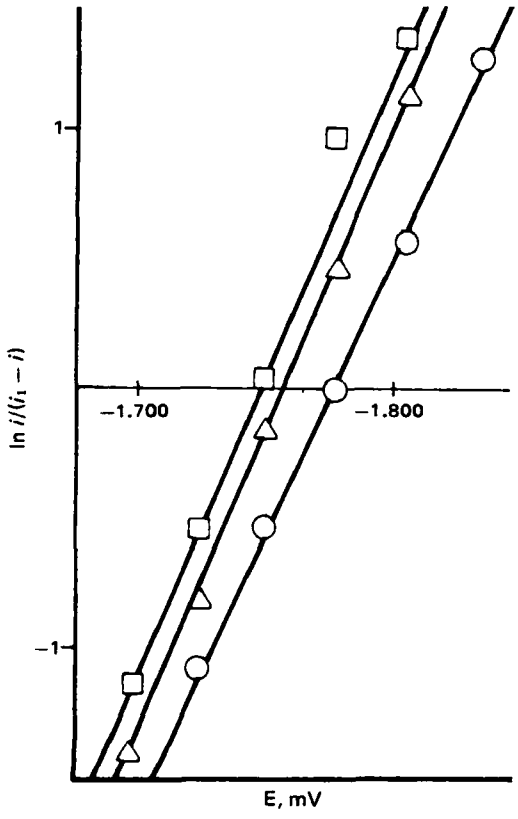

Figure 2—*Tomes representations;* i_1, *limit current; 1, DC current; E, potential* versus *Ag/AgCl, saturated lithium chloride. Key: (□) pH 6.87; (△) pH 6.79; (○) pH 6.13.*

influence was considered) as follows: dropping time, τ, 0.6 sec; height of the mercury-containing vessel, h, 54 cm; temperature, T, 25°; phenformin hydrochloride concentration, c, 3.8×10^{-4} M; the ionic strength was adjusted to μ 0.5 with 1:1 inert electrolyte; mercury flow, m, 0.88 mg/sec (at the referred height h).

A sample volume of 25 ml of freshly prepared solution was used in all of the polarographic experiments. Gelatin was used as a suppressor in a proportion of 0.01% by weight, in the polarographic recordings of phenformin only. Auxiliary and reference silver/silver chloride electrodes together with a 1:1 electrolyte-saturated salt bridge were used.

The polarographic result found by means of the DC technique agreed well with the ones found using the AC_1 technique.

Phenformin hydrochloride was used as supplied[3], and the melting point (175 ± 1°) and IR spectra were tested. Two aqueous suspensions of insulin–zinc complexes both of 40 IU/ml were used[4]: a mixture of 70% crystalline and 30% amorphous porcine and bovine and a 100% amorphous porcine insulin–zinc suspension, respectively.

RESULTS AND DISCUSSION

An irreversible cathodic polarographic wave is detected in buffered lithium–phosphate–chloride–gelatin aqueous solutions of phenformin in the pH range 4–7. This wave involves a one-electron transfer according to the slopes of Tomes representations obtained from DC data (Fig. 2). This wave does not appear in the same media in the absence of phenformin.

The peak potential, E_p, depends slightly on phenformin concentration (Fig. 3), but the height of the wave is independent of that concentration at pH values >5.5.

Such polarographic behavior implies the presence of active catalytic centers for the reduction of hydrogen ions, and more precisely it points to the existence of localized electron pairs over nitrogen on phenformin, since such active centers occur in other related substances (12).

The formation of Zn^{2+}–phenformin complexes (MF_j) has been studied by DC polarography in Britton–Robinson buffered media. Provided that the detected polarographic wave results are quasi-reversible, the procedure

[3] Funk Laboratories S. A., Barcelona, Spain.
[4] Novo Laboratories, Copenhagen, Denmark.

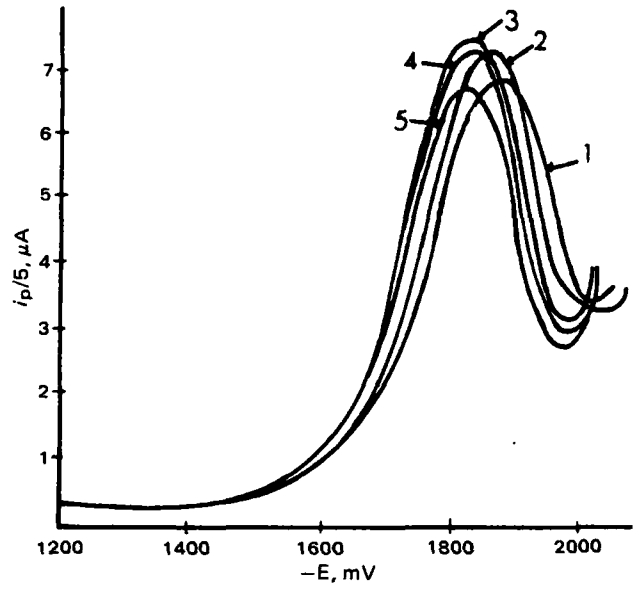

Figure 3—*Effect of phenformin concentration on AC_1 waves. Key: (1) c = 3.8 × 10⁻⁴ M; (2) c = 4.6 × 10⁻⁴ M; (3) c = 5.3 × 10⁻⁴ M; (4) c = 6.1 × 10⁻⁴ M; (5) c = 6.8 × 10⁻⁴ M. E, potential* versus *Ag/AgCl, saturate lithium chloride; ΔE = 30 mV, pH 5.40.*

developed by Lingane (13) to express the variation of half-wave potential on complexation has been used:

$$\Delta E_{1/2} = j\frac{0.0591}{n\alpha}\log c_F + \frac{0.0591}{n\alpha}\log \beta_{(MF_j)} \qquad \text{(Eq. 1)}$$

where $\Delta E_{1/2} = E^f_{1/2} - E^c_{1/2}$ expresses the difference between half-wave potentials of free and complexed zinc cation, respectively, and c_F is the ligand concentration.

The dependence of $\Delta E_{1/2}$ measured values with $\log c_F$ adjusts well to a linear equation for a concentration range of $4 \times 10^{-4} < c_F < 7 \times 10^{-4}$ M, and $[Zn^{2+}] = 2 \times 10^{-4}$ M (T = 25°; pH 4.97). From this linear dependence, a ligand number of $j = 2$ is found, and the adjusted stability constant is $\beta_{(MF_2)} \simeq 2 \times 10^{11}$ M^{-2}. [Despite the fact that the stability constant value found indicates that the phenformin–Zn^{2+} complex is a quite stable complex, it must be noted that biguanides form stronger complexes with some other metallic cations (14)].

The stoichiometry of zinc–insulin complexes is less clear. As reported previously (15), zinc ions appear to combine preferentially with imidazole groups. Several Zn^{2+}–imidazole relationships have been noted: from 1:1 complexes to 1:3 and 1:4. There is strong binding between two metal ions and three imidazole dimers, especially in crystalline metal–insulin complexes (16). These authors suggest that the first zinc ion is bound by three coordination bonds, being the association constant of the order of the ones normally found for zinc–imidazole interactions ($\simeq 10^9$), and the bonding of the second Zn^{2+} appears to be 10^4 times stronger.

Insulin binds strongly with zinc cations in both amorphous and crystalline complexes, impeding the polarographic reduction of Zn^{2+} (15). The polarographic reductions observed in the study of amorphous insulin–zinc suspensions (curve A, Fig. 4) and crystalline–amorphous mixture suspensions (curve C, Fig. 4C) are due to free zinc cations whose concentrations polarographically derived are: $[Zn^{2+}]_A = 1.64 \times 10^{-5}$ M, and $[Zn^{2+}]_C = 2.24 \times 10^{-5}$ M.

The addition of phenformin to insulin–zinc complex suspensions modifies the polarographic recordings (curves B and D, Fig. 4). In the case of the addition to amorphous complex suspension (curve B) a shift to more negative values of half-wave potential is observed, indicating that phenformin has complexed zinc cations. The increasing curve height (curve B from A) indicates that the phenformin complexed Zn^{2+} concentration (recorded in B) $[Zn^{2+}]_B = 2.1 \times 10^{-5}$ M, is greater that the previous free Zn^{2+} concentration $[Zn^{2+}]_A$. This increase in the reducible phenformin complexed Zn^{2+} concentration can only be explained if some insulin–zinc complexes have been broken by the action of phenformin.

In the case of the amorphous and crystalline insulin–zinc mixture (curves C and D, Fig. 4) a quite different behavior is observed. Also the half-wave potential is shifted to more negative values, indicating the complexation of Zn^{2+} with phenformin, but the increase in reducible Zn^{2+} concentration $[Zn^{2+}]_D = 2.36 \times 10^{-5}$ M is less than in the previous case of pure amorphous insulin–zinc suspensions. This small increase corre-

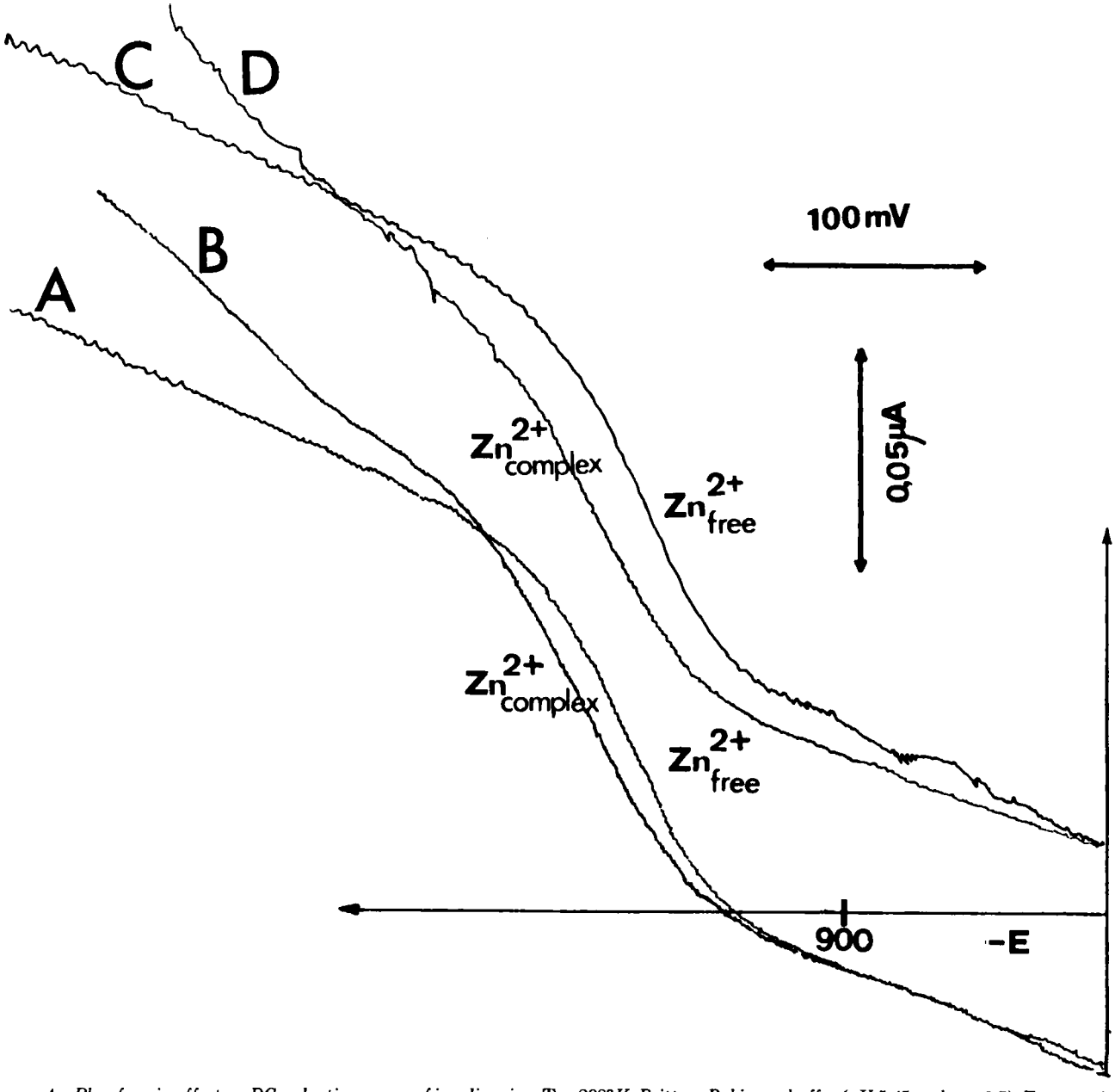

Figure 4—*Phenformin effect on DC reduction waves of insulin–zinc.* T = 298°K; *Britton–Robinson buffer (pH 5,45 and* μ = 0.5); *E* versus *Ag/AgCl, saturated potassium chloride; [Zn^{2+}] measured by DC polarography. (A) 8.366 g/liter amorphous insulin–zinc; (B) 8.366 g/liter amorphous insulin–zinc with phenformin (4 × 10^{-4} M); (C) 8.366 g/liter crystalline (70%) and amorphous (30%) insulin–zinc; (D) 8.366 g/liter crystalline (70%) and amorphous (30%) insulin–zinc with phenformin (4 × 10^{-4} M).*

sponds only to the amorphous part of the mixture as can be derived from the increase detected in the first case, indicating that the crystalline insulin–zinc complex remains stable despite the presence of phenformin.

The polarographic experiments showed that there is a competition between phenformin and insulin toward zinc cations. The antidiabetic behavior of phenformin can be related to this competition. This competition is not restricted to β-cells environment, rather it will work in any part of the living organism where these molecules meet and also can explain the protection of insulin against biochemical degradation (17).

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