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# CONFORMATIONAL ANALYSIS OF THE CALCIUM-ANTAGONIST GALLOPAMIL

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**Abstract**—Conformational analysis of gallopamil was performed in order to gain insight into the molecular determinant of its calcium-antagonistic property. Whereas the neutral form of gallopamil was characterized by a single, largely predominant configuration, the protonated form of the drugs yielded several conformers, some of which were characterized by a readily accessible ionized site. The capacity of gallopamil to inhibit ionophore-mediated calcium translocation in a two-phase bulk system was inversely related to the pH of the aqueous phase. These findings indicate that the capacity of gallopamil to interfere with the transport of cations is critically dependent on the availability of a protonated configuration of the drug.

Organic calcium-antagonists such as verapamil and gallopamil are widely used in clinical medicine and experimental biology to block $Ca^{2+}$ inflow into cell across the plasma membrane [1]. These drugs inhibit $Ca^{2+}$ inflow through both voltage-sensitive and insensitive $Ca^{2+}$ channels, as well as $Ca^{2+}$ transport mediated by ionophoretic molecules across biological or artificial membranes [2–4]. Studies performed in artificial systems have led to the concept that these antagonists compete with $Ca^{2+}$ for a common binding site on the ionophoretic molecule [5, 6]. In order to gain further insight into the mechanism of action of these calcium-antagonists at the molecular level, we have characterized, in the present study, the conformation of gallopamil (D600, see Fig. 1) by conformational analysis.

## MATERIALS AND METHODS

The method used for the conformational analysis of gallopamil is based on a strategy described elsewhere [7] and currently used for studying the conformation of peptides [8, 9] and other molecules [10–13]. The total conformational energy is calculated as the sum of the contributions resulting from the van der Waals' interaction, the torsional potential and the electrostatic interaction. The latter was calculated for a dielectric constant of 16, a value chosen to simulate the proximity of a membrane interface [11]. In a first systematic study, the eight torsional angles between the phenyl groups underwent successive increments of 120° for torsional angles 4 and 12 (see Fig. 1) and 60° for angles 6–11 yielding $6^6 \cdot 3^2$ different conformations of either the neutral or protonated form of gallopamil. The conformers derived from this first study and yielding a low internal energy, i.e. those with a statistical weight of at least 2.5%, were then submitted to a second analysis,

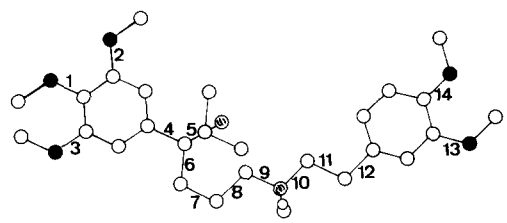


Fig. 1. Initial all-*trans* configuration of gallopamil (α-isopropyl-α[(*N*-methyl-*N* - homoveratril) - γ - aminopropyl]-3,4,5-trimethoxyphenyl-acetonitrile), with numbering of the torsional angles. Open circles refer to carbon atoms (>C<, —CH=, —$CH_2$—, —$CH_3$). Hatched and closed circles refer to N and O atoms, respectively. The C atom of the cyano radical is masked by the C atom of the isopropyl radical.

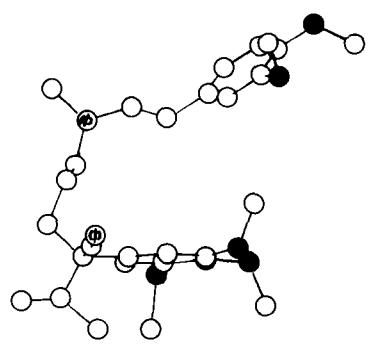

Fig. 2. Configuration of the conformer N (see Table 1) of gallopamil, in the neutral form, after function minimization. The symbols for each atom are the same as those used in Fig. 1.

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Table 1. Conformational analysis of gallopamil

| Conformer* | I | II | III | IV | V | VI | VII | VIII | N |
|---|---|---|---|---|---|---|---|---|---|
| Energy† | 0 | 0.122 | 0.139 | 0.186 | 0.624 | 0.699 | 0.818 | 1.198 | 0 |
| Probability‡ | 19.24 | 15.65 | 15.21 | 14.04 | 6.71 | 5.90 | 4.83 | 2.54 | 97.59 |
| Conformer§ | $\alpha$ | $\beta$ | $\beta$ | $\gamma$ | $\gamma$ | $\delta$ | $\varepsilon$ | § | N' |
| Angle 1‖ | 93.5 | 99.2 | 91.3 | 94.5 | 89.4 | 96.8 | 94.6 | 93.3 | 103.1 |
| 2 | 251.8 | 256.1 | 242.0 | 250.4 | 233.2 | 252.5 | 243.7 | 244.3 | 263.6 |
| 3 | 78.0 | 71.1 | 76.8 | 79.5 | 71.7 | 87.8 | 79.6 | 79.2 | 91.5 |
| 4 | 68.0 | 60.5 | 65.9 | 66.5 | 65.0 | 60.0 | 58.2 | 68.3 | 58.1 |
| 5 | 188.7 | 188.8 | 187.7 | 189.0 | 188.8 | 191.3 | 188.0 | 189.3 | 190.6 |
| 6 | 60.4 | 58.8 | 60.2 | 58.9 | 59.2 | 57.7 | 63.2 | 59.6 | 60.9 |
| 7 | 70.2 | 65.2 | 62.9 | 68.0 | 62.6 | 286.2 | 67.7 | 70.0 | 66.3 |
| 8 | 161.0 | 166.8 | 172.0 | 152.6 | 154.4 | 166.0 | 281.3 | 145.9 | 187.6 |
| 9 | 105.0 | 141.7 | 113.3 | 113.4 | 130.9 | 85.2 | 146.3 | 100.0 | 281.6 |
| 10 | 216.2 | 255.9 | 244.4 | 235.7 | 228.6 | 209.1 | 267.5 | 216.6 | 285.8 |
| 11 | 181.5 | 61.7 | 63.6 | 298.1 | 298.1 | 78.2 | 294.5 | 181.4 | 195.2 |
| 12 | 100.5 | 72.0 | 66.8 | 109.6 | 101.1 | 101.4 | 298.2 | 85.4 | 308.8 |
| 13 | 84.9 | 85.7 | 69.2 | 81.1 | 59.6 | 79.4 | 87.3 | 80.4 | 96.2 |
| 14 | 110.2 | 260.8 | 255.5 | 260.8 | 243.2 | 260.5 | 262.0 | 262.0 | 271.8 |

* Conformers I–VIII and N were derived from the analysis of the protonated and neutral forms of gallopamil, respectively.

† The energy above minimal value in the I–VIII and N series is expressed as kcal/mole.

‡ The probability of existence or statistical weight in the I–VIII and N series is expressed as per cent by application of the Boltzmann distribution law.

§ The conformers $\alpha$–§ and N' were obtained after application of the minimization procedure on conformers I–VIII and N. The same symbol is used for conformers which eventually displayed virtually similar configurations.

‖ The angles of bonds 1–14 (see Fig. 1) are expressed as degrees.

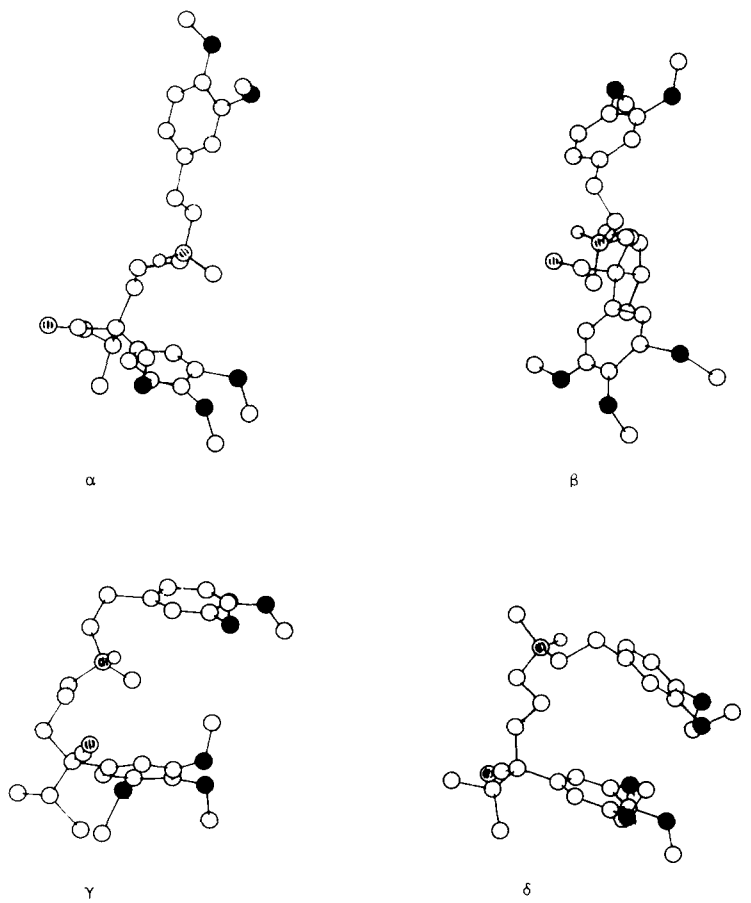


Fig. 3. Configuration of the conformers $\alpha$, $\beta$, $\gamma$ and $\delta$ (see Table 1) of gallopamil, in the protonated form, after function minimization. The symbols for each atom are the same as those used in Fig. 1.

using the simplex minimization procedure [14] in order to further reduce their total energy. Calculations were made on a CDC-Cyber 170 computer coupled to a Benson drawing table (Brussels University Computing Center).

The effect of gallopamil upon A23187-mediated calcium translocation was examined in a two-phase bulk system, as described in detail elsewhere [15]. Briefly, an aliquot (0.2 ml) of a Tris–HCl aqueous buffer (20 mM, pH 7.00–8.15) containing NaCl (120 mM) and a mixture of $^{45}CaCl_2$ and $^{40}CaCl_2$ (0.1 mM) was vigorously mixed at room temperature with an equal volume of a mixture of toluene and butanol (7/3, v/v) containing the antibiotic ionophore A23187 (5–50 $\mu$M) and, when required, gallopamil (1 mM). The amount of $^{45}Ca$ translocated in the organic phase was judged from the radioactive content of the organic phase.

## RESULTS

### *Conformational analysis*

The chemical structure of gallopamil is illustrated in Fig. 1, which also indicates the numbering of the 14 torsional angles in the molecule. The systematic study performed on the neutral form of gallopamil, yielded one conformer (N) with a 97.6% probability. Fig. 2 illustrates this conformer after application of the minimization procedure.

The systematic study performed on the protonated form of gallopamil yielded among 419,904 possibilities eight conformers (I–VIII) with an individual probability of more than 2.5% (Table 1) and an integrated probability of 84.1%. After application of the minimization procedure, these eight conformers yielded six distinct conformations ($\alpha$–§) defined in Table 1. Indeed, after minimization, conformers II and III yielded conformations virtually superimposable. Such was also the case for conformers IV and V. Fig. 3 illustrates four conformations of the protonated form of gallopamil corresponding to the most probable conformers in this series.

### *Calcium-antagonistic property*

In order to assess the influence of pH, and hence gallopamil ionization, on its calcium-antagonistic capacity, we have characterized the inhibitory effect of the drug upon ionophore-mediated calcium translocation in a two-phase bulk system, the pH of the aqueous phase varying between the extreme values of 7.00 and 8.15. As shown in Fig. 4 (upper panels), gallopamil, at a fixed concentration of 1 mM (initial concentration in the organic phase), markedly inhibited A23187-mediated Ca translocation when the pH of the aqueous phase amounted to 7.00, but failed to do so at a higher pH (8.15). At neutral pH, the inhibitory effect of gallopamil was evident whatever the concentration of ionophore (5–50 $\mu$M initial concentration in the organic phase). In the absence of gallopamil, the translocation of calcium increased as a power function of the ionophore concentration (Fig. 4, middle left panel) or pH of the aqueous phase (Fig. 4, middle right panel). The dependency on A23187 concentration of the translocation phenomena was not vastly different at pH ranging

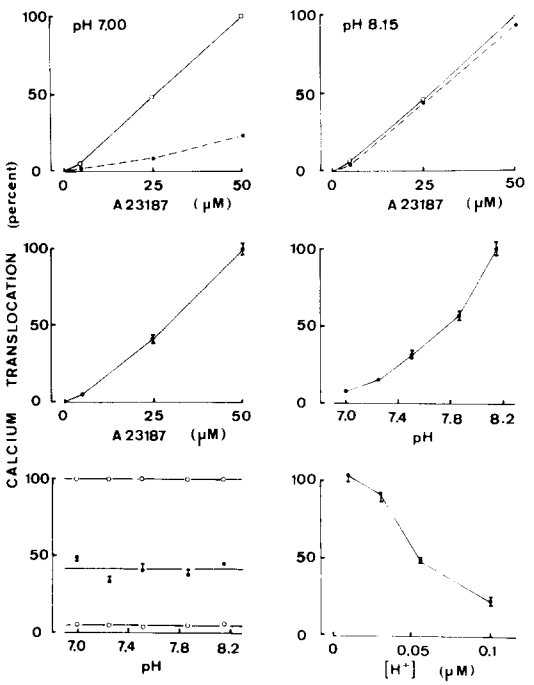


Fig. 4. Effect of gallopamil and A23187 upon Ca translocation in a two-phase bulk system. Upper panels: effect of increasing concentrations of A23187 upon Ca translocation in the absence (open circles and solid line) or presence (closed circles and dotted line) of gallopamil (1 mM), the pH of the aqueous phase amounting to 7.00 (left) or 8.15 (right). Middle panels: effect of increasing concentrations of A23187 (left) and increasing pH (right) upon Ca translocation in the absence of gallopamil. Lower panels: effect of pH upon A23187-mediated Ca translocation in the absence of gallopamil (left) and at increasing concentrations of A23187 (from bottom to top: 5, 10 and 50 $\mu$M) and in the presence of 1 mM gallopamil (right). All results are expressed as per cents of the appropriate control value found in the absence of gallopamil. In the upper panels, this control value refers to the data collected at the same pH in the presence of 50 $\mu$M A23187. In the middle panel, the control value refers either to the higher concentration of A23187 (left) or higher pH (right), data obtained at different pH (left) or concentrations of A23187 (right) having eventually been pooled together. In the lower panels, the control value refers to the data obtained in the sole presence of A23187 at the same pH (or $H^+$ concentration), the experimental data collected in the presence of gallopamil (right) representing the average value of measurements performed at three concentrations of A23187 and this at each $H^+$ level. Mean values (± S.E.M.) refer to three or more individual determinations.

between 7.00 and 8.15 (Fig. 4, lower left panel). However, in contrast with the latter behaviour, the relative magnitude of the inhibitory action of gallopamil was dramatically dependent on pH, averaging 76.9 ± 2.6% at pH 7.00, 51.1 ± 1.4% at pH 7.25, 8.3 ± 6.3% at pH 7.51, and being completely abolished at pH values of 7.87 or 8.15 (Fig. 4, lower right panel). In none of these experiments was the ionophoretic capacity of A23187 fully saturated. Thus, even in the presence of 50 $\mu$M A23187 and at pH 8.15, the apparent concentration of Ca in the organic phase did not exceed 15.1 ± 0.3 $\mu$M, as

distinct from a saturation value of 25 $\mu$M [15]. This situation is attributable to the low concentration of $Ca^{2+}$ (0.1 mM) present in the initial aqueous phase [15].

## DISCUSSION

The conformational analysis of the calcium-antagonist gallopamil indicates that the neutral form of the drug is characterized by a unique conformation, whereas the protonated form exists in different conformations with great mobility of the torsional angles and of the ionized site of the molecule. Among the latter several conformers, conformations $\gamma$ and $\delta$ (see Table 1 and Fig. 3) were characterized by an easily accessible cationic site and, hence, could be considered as most suitable for interaction with the calcium-binding site of ionophoretic systems. If these conformations were to represent the biologically active species, their selective binding to the cationic transporting system(s) would allow for their further generation from other conformers as long as equilibrium is not reached between the bound and free molecules of the biologically active species.

The functional importance of the protonated site on the gallopamil molecule is supported by our observation that the inhibitory effect of the drug upon ionophore-mediated calcium translocation was more marked at low than at high pH, within a range of values close to that found in biological fluids. This finding may have physiological implications. For instance, since organic calcium-antagonists inhibit the ionophore-mediated translocation of both monovalent and divalent cations [6], the present finding could account, in part at least, for the sensitivity to organic calcium-antagonists of the process of $K^+$ passive outflow from cells along its electrochemical gradient [16]. Indeed, when inserted in the phospholipid domain of the plasma membrane and forming a complex with native ionophores, gallopamil would be optimally placed to inhibit $K^+$ extrusion since the binding of this cation to the postulated carrier would occur at the inner phase of the plasma membrane, i.e. at the relatively low pH characteristic of the cytosolic compartment.

In conclusion, both the conformational and ionophoretic data presented in this report indicate that the capacity of gallopamil to interfere with the transport of cations may be critically dependent on the existence and availability of a protonated configuration of the drug.

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