Physicochemical properties of the H_2-receptor antagonist cimetidine

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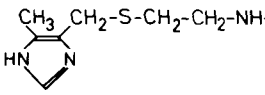
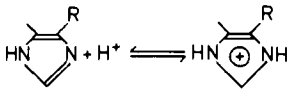
Cimetidine (1-cyano-2-methyl-3-[2-(5-methylimidazol-4-ylmethylthio)ethyl]guanidine), is a potent H_2-



(1)

receptor antagonist characterized by the electron withdrawing sidechain R on the imidazole ring. As a consequence, the protonation of cimetidine is given by the equilibrium:



abbreviated to: cimN + H^+ ⇋ cimNH^+. For this system the pK_a equals 7·09 at 25 °C in 0·1 M NaCl (Durant et al 1977; Ganellin 1978).

To obtain more information about the mechanism of cimetidine's H_2-receptor antagonism, we have examined its effect on surface adsorption, surface tension and surface potential. In addition bulk properties were investigated by differential refractometry. The drug was studied in aqueous solution at different pH values, as function of temperature and concentration.

Tensioactivity and surface adsorption

The equation of Szyskowski (1908) as modified by Meissner & Michaels (1949) relates the surface tension σ to the concentration c of solute in the bulk solution:

$$\sigma = \sigma_0 - RT\,\Gamma^\infty \ln(1 + C/\beta) \quad .. \quad .. \quad (1)$$

where σ_0 is the surface tension of the solvent, R the molar gas constant, T the absolute temperature, Γ^∞ the total surface concentration of solute per unit area at saturation and β the constant in the Langmuir equation. Equation (1) may be rewritten in terms of the surface pressure π:

$$\pi = \sigma_0 - \sigma = RT\,\Gamma^\infty \ln(1 + C/\beta) \quad .. \quad (2)$$

With allowance for the dissociation equilibrium of cimetidine, the surface pressure is:

$$\pi = RT\,\Gamma^\infty \ln\left(1 + \frac{C_2}{\beta_2} + \frac{C_3}{\beta_3}\right) \quad .. \quad (3)$$

where subscript 2 and 3 relate to cimN and cimNH^+ respectively. This is equivalent to equation (2) if $c = c_2 + c_3$, $\beta = \beta_2\,\beta_3/(\beta_2 + k\,\beta_3)/(1 + k)$ and $k = K_a/C_{H^+} = C_2/C_3$.

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Surface tension, measured in a glass cell, thermostated at 37·0 °C ± 0·1 °C, by means of an electro balance type Cahn RG, decreased slowly as a function of time, tending to an equilibrium value after 3 h. The decrease is probably due to reorientation of the adsorbed molecules. Fig. 1 shows this for an aqueous solution of 10^{-2} and 1·8 10^{-2}m cimetidine at pH 4·5 and 37 °C.

The Gibbs isotherm in stable equilibrium (after 3 h) at a pH 4·5 and 37 °C in 0·1 M NaCl solution allowed the calculation of the minimum molecular surface of $A_0 = 66{\cdot}10^{-2}$ cm². Using the values of the internuclear distances and of the valency angles, the theoretical molecular area A_0 of the molecule can be calculated. When the imidazole ring is oriented to the water phase, and the side chain R is parallel to the interface, the theoretical value $A_0 = 72{\cdot}10^{-16}$ cm² is in good agreement with the experimental value.

Surface potential

As the polar molecules are adsorbed at an interface, a potential difference between the surface and the bulk of the system is built up. This surface potential is directly related to the component of the molecular dipole moment, perpendicular to the interface.

A change of the surface potential results from an alteration in the adsorption or from a modification of the perpendicular component of the molecular dipole moment. The latter is due either to a reorientation of the molecules at the interface or to a transformation in the structure of the adsorbed molecules.

The surface potential was determined according to Kenrick (1896), with the measuring cell as constructed in our laboratory (Fig. 2). From the cell A, containing the solution of cimetidine, a central jet flows out of the

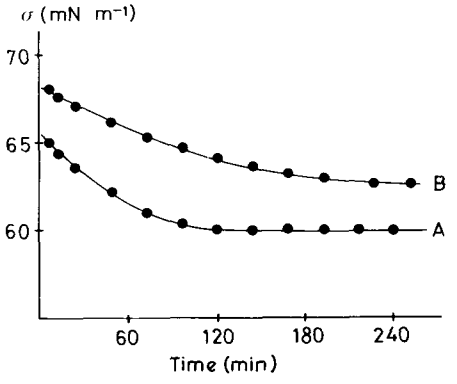

FIG. 1. Variation of the surface tension as function of the time for two different concentrations of cimetidine in 0·1 M NaCl at pH 4·5 and 37 °C. A. 1·8 10^{-2} M B. 2·0 10^{-2} M.

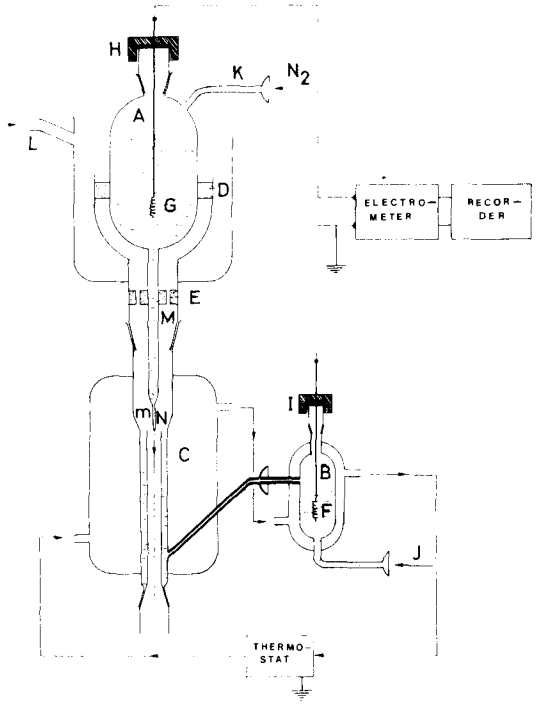

FIG. 2. Cell for surface potential measurements. A. cell B. overflow C. coaxial cylinder m. capillary.

capillar m (length 2 cm, i.d. 0·007 cm) into the coaxial cylinder C. At the same time the system B, comprising the reference solution without cimetidine, feeds cylinder C via the overflow M. In this way a laminar film is built up on the inside of cylinder C.

The streaming velocity of the central jet and of the overflow are regulated independently by applying pressure with nitrogen. The whole system is thermostated within 0·2 °C.

The surface potential can be written as (Vochten & Petré 1975):

$$\chi^{I} - \chi^{II} = \Delta V + \frac{RT}{F} \ln \frac{a^{I}_{Cl^-}}{a^{II}_{Cl^-}} \quad \ldots \quad (4)$$

where F is the Faraday constant, $a^{I}_{Cl^-}$ and $a^{II}_{Cl^-}$ respectively are the activities of the Cl^- ions in the solution of the cell A(I) and in the overflow B(II). In our experimental situation, the concentration of NaCl in the system was 0·1 M NaCl so that $a^{I}_{Cl^-} = a^{II}_{Cl^-}$. As a consequence the surface potential $\Delta\chi = \chi^{I} - \chi^{II}$ (4) was given by the potential difference ΔV measured between G (cell A) and F (overflow B). G and F being two identical Ag/AgCl electrodes. To obtain reproducible results, all the solutions were saturated with solid AgCl.

The potential difference V was measured with an electrometer type Carry 401 combined with a Kipp BD-5 recorder. The measuring cell A and the cylinder system C were placed in a Faraday cage.

Using solutions of cimetidine freshly prepared daily, the surface potential was measured as a function of concentration and temperature. Initially the value of the surface potential as a function of the N_2-pressure, applied on the central jet was investigated. In the experimental range, the potential was independent of the streaming velocity of the jet, demonstrating that cimetidine is adsorbed rapidly, the time of adsorption being less than 10^{-3}s. This process was finished before the reorientation of the molecules occurred.

The surface potential as a function of the concentration of cimetidine is shown in Fig. 3. From a comparison with a similar study on alcohols (Vochten & Petré 1971; Vochten 1976) we conclude that this augmentation is due to the greater adsorption of cimetidine molecules at the interface which results directly in an increase of the cumulative total dipole moment.

The surface potential measured as a function of temperature at two different concentrations at pH 4·5 (Fig. 4) shows a sharp minimum at 37 °C.

The diminution of the surface potential from 20 to 37 °C corresponds with the expected behaviour resulting from a decrease in adsorption at the interface. The increase with temperatures higher than 37 °C reflects an increase in the total dipole moment, probably as a direct consequence of the dependence of the dissociation constant on temperature. According to Datta & Grzybowski (1966), the acidity constant of the imidazolium cations increases with increasing temperature. This means that increase in temperature will reduce the proportion of protonated cimetidine. As a consequence, the substitution of cimetidine in the cation form by the molecules causes a larger total dipole moment.

Differential refractometry

The differential refractive index as a function of temperature and concentration was investigated to

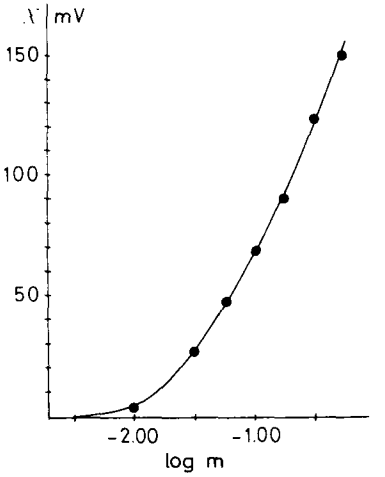

FIG. 3. Variation of the surface potential as function of concentration of cimetidine in 0·1 M NaCl at pH 4·5 and 37 °C.

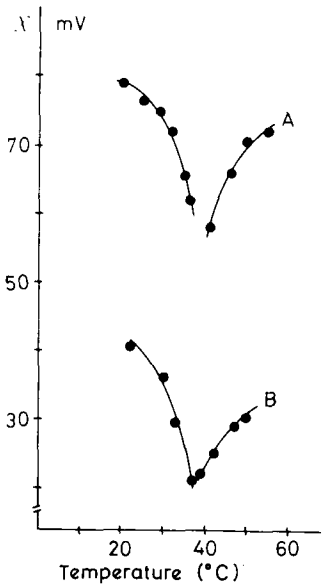

FIG. 4. Variation of the surface potential as function of temperature for two different concentrations of cimetidine in 0·1 M NaCl at pH 4·5. A. $1·0\ 10^{-1}$ M B. $3·2\ 10^{-2}$ M.

analyse a typical property of the bulk of the solution. The apparatus used was a Brice-Phoenix differential refractometer type 1120, in which the closed measuring cell was connected to an ultrathermostat accurate to 0·02 °C. The measurements were at 436 nm.

In Fig. 5, the differential refractive index is shown as a function of concentration at 37 °C. These measurements demonstrate that there is no formation of micelles in the solution.

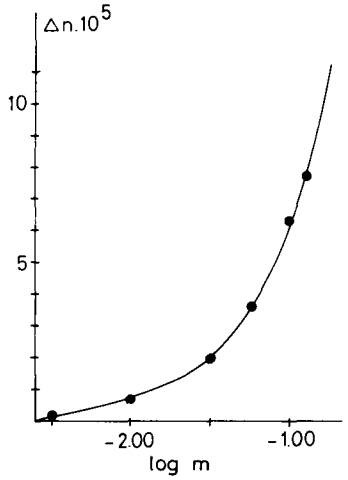

FIG. 5. Differential refractive index as function of the concentration of cimetidine in 0·1 M NaCl at pH 4·5 and 37 °C.

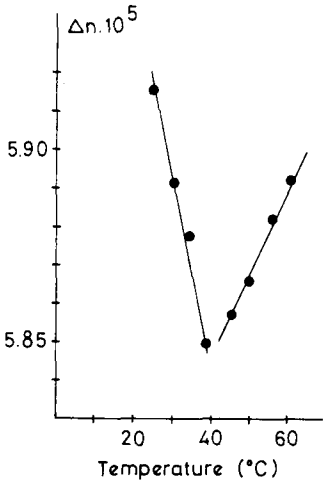

FIG. 6. Variation of the differential refractive index as function of temperature for a concentration of 1·0 10^{-1} M cimetidine in 0·1 M NaCl at pH 4·5.

The differential refractive index as a function of temperature is shown in Fig. 6 (concentration: 10^{-1}m). From these measurements, we conclude that the behaviour of the surface phenomenon reflects in the physicochemical properties of the bulk. This demonstrates that the minimum in the surface potential is directly related to a typical bulk effect.

Conclusion

The measurements show that the surface and the bulk properties have a typical related behaviour as function of temperature, probably due to the degree of protonation of the cimetidine molecule in solution. As is known, a potential barrier is present in the neighbourhood of the muscosa of the stomach. This barrier repels the protons into the stomach. As cimetidine is adsorbed rapidly and the surface potential at 37 °C has a minimum value, the penetration of the potential barrier by cimetidine molecules will be facilitated.

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Inhibition of the release of slow-reacting substance of anaphylaxis by inhibitors of lipoxygenase activity

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Recent evidence has suggested that both 5-hydroxy-6-glutathionyl-eicosatetraenoic acid (leukotriene C) and its probable degradation product 5-hydroxy-6-cysteinyl-glycine eicosatetraenoic acid (leukotriene D) contribute to the biological activity hitherto referred to as slow-reacting substance of anaphylaxis (SRS–A) (Orning et al 1980; Morris et al 1980b). These compounds are believed to derive from 5-hydroperoxy-eicosatetraenoic acid (5-HPETE) produced from the oxygenation of arachidonic acid by a lipoxygenase enzyme (Samuelsson et al 1979).

The possibility of a pivotal role for lipoxygenase activity with regard to SRS–A production has received indirect support from experimental results using non-steroidal anti-inflammatory drugs (NSAIDs). In general, these drugs enhance SRS–A release (Walker 1973; Engineer et al 1978), and it has been suggested that this effect is related to inhibition of prostaglandin (PG) biosynthesis which in turn controls SRS–A production. More recent evidence, however, with drugs such as steroids and eicosatetraynoic acid which inhibit both PG biosynthesis and SRS–A production, has suggested that the enhancement observed with NSAIDs may be due to diversion of arachidonate down a lipoxygenase mediated pathway (Burka & Flower 1979; Hitchcock 1978; Morris et al 1980a).

Benoxaprofen, a new anti-inflammatory agent, has recently been reported to inhibit the production of hydroxy-acid derivatives of arachidonic acid by rabbit PMN lipoxygenase enzymes (Walker & Dawson 1979), whilst possessing relatively low PG cyclo-oxygenase inhibitory activity (Cashin et al 1977). This communication compares the effect of benoxaprofen with those of BW755c, another lipoxygenase inhibitor (Higgs et al 1979), and with two potent PG cyclo-oxygenase inhibitors, indomethacin and piroxicam (Carty et al 1980) on the formation of both lipoxygenase products and SRS–A. The effect of nordihydro-guaiaretic acid (NDGA) a free radical scavenger and known lipoxygenase inhibitor (Hamberg 1976) was also assessed.

Inhibition of lipoxygenase activity was demonstrated using intact elicited rabbit peritoneal polymorpho-nuclear leucocytes (PMNs) as previously described (Walker & Dawson 1979). Briefly, isolated cells were exposed to calcium ionophore, A23187, in the presence of [1-^{14}C]arachidonic acid after 10 min preincubation with or without compounds at the concentrations indicated. Incubations were terminated after 5 min and the acidic lipids extracted and subjected to radio-t.l.c. and quantitation as previously described.

The release of SRS–A from guinea-pig chopped lung was assessed as described elsewhere (Dawson & Sweatman 1980). Briefly, lungs excised from previously sensitized guinea-pigs were perfused with Tyrode solution and chopped into small cubes. Samples of tissue were incubated with or without drug for 5 min before challenge with ovalbumin. Supernatants were removed after 15 min incubation at 37 °C and bio-assayed for SRS–A activity using a mepyraminized guinea-pig ileum preparation. Compounds were tested at the highest concentrations used for direct antagonism of the 'in house' guinea-pig SRS–A standard.

Both benoxaprofen and BW755c inhibited hydroxy-acid and SRS–A formation in a concentration dependent fashion. NDGA, however, while effectively inhibiting the rabbit lipoxygenase, failed to modify the production of SRS–A except at the highest concentration used. Lower concentrations of the drug resulted in non-concentration dependent potentiation (Table 1).

At concentrations higher than those required to inhibit PG biosynthesis, both indomethacin and piroxicam inhibited SRS–A release but not hydroxy-acid formation. At lower concentrations indomethacin potentiated both SRS–A and hydroxy-acid formation, whereas piroxicam did not. None of the drugs examined affected the response of the ileum to guinea-pig SRS–A standards.

The results described provide direct evidence for the involvement of a lipoxygenase pathway in the biosynthesis or release of SRS–A. Benoxaprofen appears to be a slightly more potent inhibitor on the lung system than on the PMNs (IC50 lung, 10^{-5} M; IC50 PMN, 6×10^{-5} M, Walker & Dawson 1979). BW755c, a structural analogue of phenidone, inhibits PG cyclo-oxygenase and horse platelet lipoxygenase activity at similar concentrations (Higgs et al 1979). In the present study the drug has been found to be inhibitory for both

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