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Substituted benzimidazoles inhibit gastric acid secretion by blocking $(H^+ + K^+)$ATPase

Erik Fellenius*, Thomas Berglindh†, George Sachs‡, Lars Olbe§, Berit Elander*, Sven-Erik Sjöstrand* & Björn Wallmark*

* Research Laboratories, AB Hässle, S-43183 Mölndal, Sweden
† Department of Physiology and Medical Biophysics, Biomedical Centre, Uppsala University, S-75105 Uppsala, Sweden
‡ Laboratory of Membrane Biology, University of Alabama in Birmingham, Birmingham, Albama 35294, USA
§ Sahlgrenska Hospital, Surgical Clinic II, S-41345 Gothenburg, Sweden

Studies both *in vivo*[1,2] and *in vitro*[1–5] have shown that substituted benzimidazoles inhibit the stimulation of acid secretion produced by dibutyryl cyclic AMP and histamine. Furthermore, the results differ from those produced by H_2 antagonists and anticholinergic agents in that the inhibition is not competitive, and the site of action is intracellular and peripheral to that of dibutyryl cyclic AMP. To investigate the biochemical mechanism of action of substituted benzimidazoles, one such compound, H 149/94 (2-{ [2-(3-methyl)pyridyl-methyl]-sulphinyl}-5-methoxycarbonyl-6-methylbenzimidazol), has been tested either directly on an $(H^+ + K^+)$ATPase isolated from pig and human gastric mucosa or on the function of this enzyme in gastric glands isolated from rabbit and human gastric mucosa. $(H^+ + K^+)$ATPase[6,7], which has only been found at the secretory surface of the parietal cell[8], catalyses a one-to-one exchange of protons and potassium ions[9–11]. It is possibly the proton pump within the gastric mucosa, and may thus be the terminal or one of the terminal steps of the acid secretory process[12,13]. We show here that H 149/94 inhibits $(H^+ + K^+)$ATPase, which may explain its inhibitory action on acid secretion *in vitro* and *in vivo*. Because of the unique distribution and properties of the $(H^+ + K^+)$ATPase, the inhibitory action of H 149/94 on this enzyme may be a highly selective clinical means of suppressing the acid secretory process.

Isolated gastric glands have been shown to respond to a number of different secretagogues and inhibitors[5,14,15]. Secretagogues such as dibutyryl cyclic AMP and K^+ stimulate acid secretion intracellularly, presumably at a site close to the $(H^+ + K^+)$ATPase[16–18]. In contrast, histamine and acetylcholine have a site of action further removed from the enzyme[14–16,19]. This may be either extracellular or at an earlier stage in the sequence of events leading to acid secretion, possibly reflecting the physiological role of such agonists in the control of acid secretion. Thus, isolated gastric glands, used in conjunction with specific cholinergic and histaminergic antagonists, provide a useful experimental model for determining the site of action of drugs influencing acid secretion, independently of hormonal and nervous influence. Although the rate of acid secretion cannot be measured quantitatively with isolated glands, the acid secretory response can be monitored semiquantitatively by measuring the uptake of a radiolabelled weak base, ^{14}C-aminopyrine (^{14}C-AP), which accumulates in the glands in proportion to the *p*H differences between the intraglandular acid compartments and the surrounding medium[14,15].

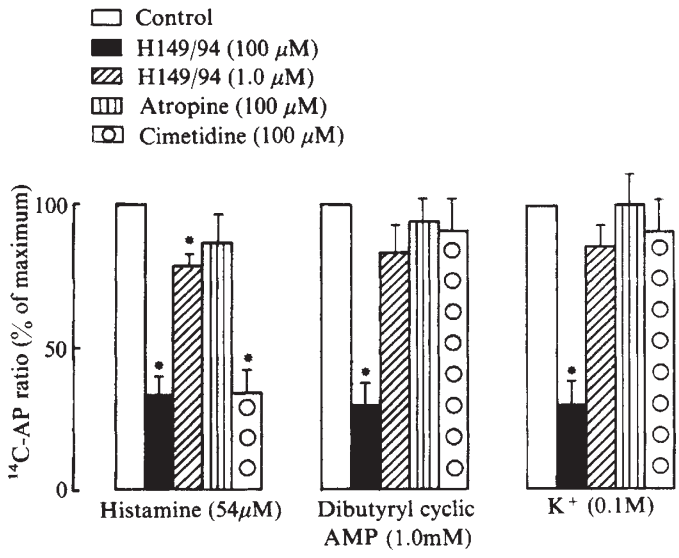

Fig. 1 The inhibitory action of H 149/94, atropine and cimetidine on ^{14}C-AP accumulation induced by different agonists in isolated gastric glands from rabbit mucosa. The preparation and incubation of the isolated glands was essentially as described elsewhere[14,15] except that the incubation volume was reduced from 3 to 0.2 ml and the amount of glands also reduced proportionally. The glands were suspended in a physiological buffer solution containing rabbit albumin (2 mg ml^{-1}), glucose (10 mM) and ^{14}C-AP (0.25 μCi and 8 μmol per incubation), and incubated in a shaking bath for 90 min at 37 °C. After the incubation the glands were spun down at 2,000*g* for 2 min. The supernatant was removed and the wet and dry weights of the pellet determined. The dried pellets were solubilized in 1 ml Soluene. Dimilume (10 ml) was added and the radioactivity then counted. H 149/94 was dissolved in methanol. All incubations contained 1% methanol, which had no influence on the ^{14}C-AP ratio. All other added substances were dissolved in the physiological buffer solution. In the experiments with high $[K^+]$ (0.1 M), the glands were washed three times with an incubation medium in which KCl (0.1 M) had been substituted for NaCl (0.1 M). The respective control values without antagonist have been set to 100%. The results are mean ±s.e. of 6–8 observations. A statistically significant difference ($P < 0.05$ Student's *t*-test) from control is denoted by an asterisk.

The effects of H 149/94 (10^{-4}, 10^{-6} M), cimetidine (10^{-4} M) and atropine (10^{-4} M) on ^{14}C-AP accumulation in rabbit gastric glands stimulated by histamine, dibutyryl cyclic AMP and K^+ are shown in Fig. 1. Atropine had no inhibitory effect in the presence of any agonist. Cimetidine inhibited the response to histamine (54 μM) but had no effect in the presence of dibutyryl cyclic AMP (1 mM) or K^+ (0.1 M). H 149/94 produced dose-dependent inhibition ($ED_{50} \sim 10^{-5}$ M) of ^{14}C-AP accumulation irrespective of the agonist used. The presence of potassium is an absolute requirement for acid secretion, presumably reflecting its involvement in the exchange process catalysed by $(H^+ + K^+)$ATPase[17,18]. The marked inhibition of K^+-induced ^{14}C-AP accumulation produced by H 149/94 therefore strongly supports the view that this compound interferes with acid secretion at or close to the enzyme, the probable terminal step of the secretory process. The lack of effect with cimetidine and atropine suggests a more distant site of action of these compounds.

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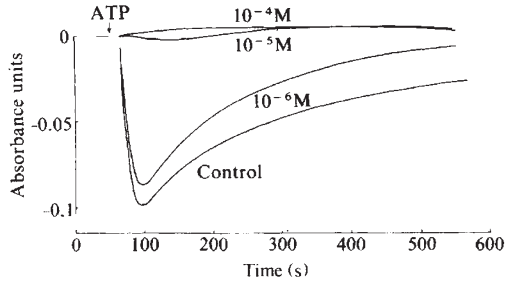

Fig. 2 Effect of H 149/94 on proton transport catalysed by the $(H^+ + K^+)$ATPase. Gastric membranes were prepared from pig stomach by differential and zonal density gradient centrifugation[20]. The vesicle fraction (~40 μg ml^{-1}) was equilibrated at 4 °C overnight in the following medium: 2 mM PIPES-Tris, *p*H 6.7, 150 mM KCl, 2 mM $MgCl_2$ with or without H 149/94 at concentrations of 10^{-4}, 10^{-5}, 10^{-6} M as indicated. H 149/94 was dissolved in methanol. All incubations contained 1% methanol, which had no influence on the assay. At the start of the experiment 20 μM acridine orange was added to the sample and the reaction started by the addition of 0.6 mM ATP (disodium salt). The acridine orange absorbance change was monitored in an Aminco DW-2 spectrophotometer in the dual-beam mode set at 490 and 530 nm.

To explore the possibility that H 149/94 interacts directly with $(H^+ + K^+)$ATPase, the enzyme was isolated from pig stomach by differential and zonal gradient centrifugation[20]. The vesicular fraction sedimenting between the 0.25 M sucrose–8% Ficoll interface was either used directly for proton transport studies, or further purified by free-flow electrophoresis and subsequent lyophilization for studies with an ATPase assay. Fresh vesicles are relatively impermeable to cations such as protons and K^+ and readily allow measurements of proton transport[9], which can be quantified using the dye acridine orange. The control curve in Fig. 2 shows the acridine orange absorbance, when ATP is added to the K^+-preloaded vesicles in the absence of inhibitor. The interpretation of the control curve has been described elsewhere[11,21] but briefly, on addition of ATP there is a rapid loss of acridine orange absorbance corresponding to transport of H^+ into the vesicle. This is followed by an increase in the absorbance produced by an outward H^+ leak, due to depletion of intravesicular K^+. Thus, the H^+ transport signal is both ATP and K^+ dependent. In this experiment (Fig. 2) the vesicle preparation was incubated with H 149/94 at concentrations of 10^{-6}, 10^{-5} and 10^{-4} M. There was progressive inhibition of the transport signal with increasing H 149/94 concentrations—that is, proton transport was inhibited.

To test whether this inhibition of proton transport was a consequence of decreased $(H^+ + K^+)$ATPase pump activity, K^+-ATPase activities were measured at different H 149/94 concentrations. Figure 3 shows that H 149/94 produces dose-dependent inhibition of $(H^+ + K^+)$ATPase activity in contrast to cimetidine and atropine. We therefore conclude that the inhibition of proton transport by H 149/94 (Fig. 2) corresponds to the inhibition of $(H^+ + K^+)$ATPase activity seen in Fig. 3.

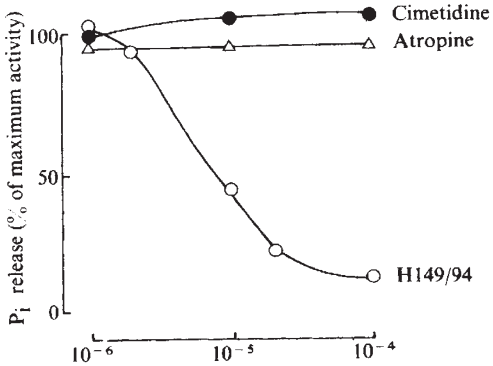

Fig. 3 The effects of H 149/94, atropine and cimetidine on isolated gastric $(H^+ + K^+)$ATPase from pig mucosa. The $(H^+ + K^+)$ATPase was obtained by differential and zonal density discontinuous gradient centrifugation followed by free-flow electrophoresis[20]. $(H^+ + K^+)$ATPase activity was quantified by the K^+-stimulated release of phosphate[22]. The incubation concentrations were Tris-HCl 40 mM, *p*H 7.4, $MgCl_2$ 2 mM, ATP 2 mM, with or without KCl 10 mM. The enzyme (~10 μg per incubation) was preincubated with the drugs for 30 min and the time of incubation was 15 min at 37 °C. H 149/94 was dissolved in methanol. All incubations contained 1% methanol, which had no influence on $(H^+ + K^+)$ATPase activity.

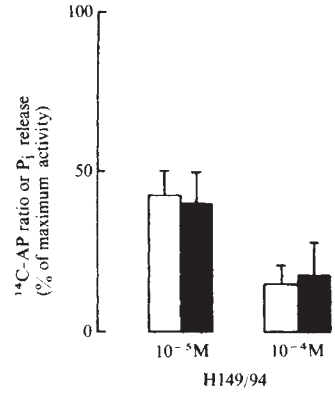

Fig. 4 The inhibitory action of H 149/94 on dibutyryl cyclic AMP (10^{-3} M)-induced ^{14}C-AP accumulation in human isolated gastric glands (□) and P_i release catalysed by isolated human $(H^+ + K^+)$ATPase (■). The mucosa was obtained from surgical biopsies taken during gastrectomy for either recurrent gastric ulcer or recurrent ulceration after previous vagotomy. The isolated glands[5] were prepared as for the isolated gastric glands from rabbit (Fig. 1). $(H^+ + K^+)$ATPase was prepared by differential centrifugation and further purified on a discontinuous step gradient[5]. The ATPase assay was performed as for pig $(H^+ + K^+)$ATPase (Fig. 3) except that the release of radiolabelled phosphate from [$\gamma - ^{32}$P]ATP was measured[23]. The results are mean ± s.e. of four observations.

H 149/94 inhibits gastric acid secretion in man (Olbe *et al.*, unpublished observation). As the therapeutic value of the compound may be its antisecretory activity in peptic ulcer patients, a series of experiments similar to those described above was performed on isolated gastric glands and $(H^+ + K^+)$ATPase obtained from gastric mucosal biopsy specimens from such patients. The results are shown in Fig. 4. H 149/94, at concentrations comparable with those found effective in the animal preparations, produced dose-dependent inhibition of both dibutyryl cyclic AMP-induced ^{14}C-AP accumulation in gastric glands, and P_i release from vesicles.

Thus, these results suggest that the substituted benzimidazole, H 149/94, is an inhibitor of the $(H^+ + K^+)$ATPase and provide a mechanism for the inhibitory effects of H 149/94 on acid secretion which have been demonstrated *in vitro* and *in vivo*[1-5]. Because of the unique distribution of the $(H^+ + K^+)$ATPase, the inhibitory effects of H 149/94 may offer a highly selective means of suppressing secretion for therapeutic purposes.

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Location of hexane in lipid bilayers determined by neutron diffraction

Stephen H. White*†, Glen I. King* & James E. Cain ‡§

*Department of Physiology and Biophysics, University of California, Irvine, California 92717, USA and the Biology Department, Brookhaven National Laboratory.
‡Biology Department, Brookhaven National Laboratory, Upton, New York 11973, USA

Knowledge of the interactions of alkanes and other small hydrophobic molecules dissolved in lipid bilayers is important for understanding lipid–protein interactions in membranes[1], the microscopic properties of solutions[2,3] and the mechanism of anaesthesia[4–6]. An essential element for describing these interactions is the distribution of the molecules across the thickness of the bilayer. Studies of black lipid films[1,2,7–9] strongly suggest that dissolved alkanes are located primarily in the centre of the bilayer. Recent X-ray and neutron diffraction and thermodynamic measurements on lipid dispersions and oriented lipid multilayers are consistent with this view[10–12]. We present here the first direct evidence for this hypothesis obtained from neutron diffraction studies of oriented dioleoyl lecithin (DOL) multilayers containing deuterated hexane introduced using the vapour phase. The hexane is found mainly in a zone 10 Å wide in the centre of the bilayer. We have also estimated the amount of hexane in the bilayers and the free energy of transfer from pure hexane to bilayer. Our numbers are considerably different from those reported by Simon *et al.*[13] for the interaction of hexane with DOL liposomes. The differences may be due to difference in water activity and bilayer curvature[14].

The neutron scattering length of the hydrogen nucleus is $-0{\cdot}38 \times 10^{12}$ cm whereas for the deuterium nucleus it is $+0{\cdot}65 \times 10^{12}$ cm (ref. 15). Consequently, the introduction of deuterated alkane into a bilayer containing non-deuterated acyl chains produces large changes in the neutron scattering density of the hydrophobic core of the bilayer. The location of the alkane can be established in the following way. Diffraction measurements are performed on lipid bilayers containing hydrogenated alkane (H-alkane) and on bilayers containing deuterated alkane (D-alkane) at the same concentration. The resulting structure factors are used to construct neutron scattering density profiles $\rho_H(x)$ and $\rho_D(x)$ of the bilayers containing, respectively, H-alkane and D-alkane. Subtraction of the two profiles yields the difference scattering density profile $\Delta\rho(x) = \rho_D(x) - \rho_H(x)$. The contribution made to each profile by the water, polar groups and acyl chains are the same and cancel out. Therefore, $\Delta\rho(x)$ represents the distribution of the deuterated alkane across the thickness of the bilayer. This general method[16] has been used to locate water[17,18], cholesterol[18] and specific atoms on phosphatidylcholine molecules[19–21] in lipid bilayers.

Here we have measured the distribution of *n*-hexane in DOL bilayers at relatively low hexane concentrations. Hexane was chosen for this initial study of alkane–bilayer interactions because (1) it has a high vapour pressure and can be introduced into the bilayer via the vapour phase in equilibrium conditions; (2) the vapour pressure can be controlled with hexane/hexadecane mixtures whose thermodynamic properties are well

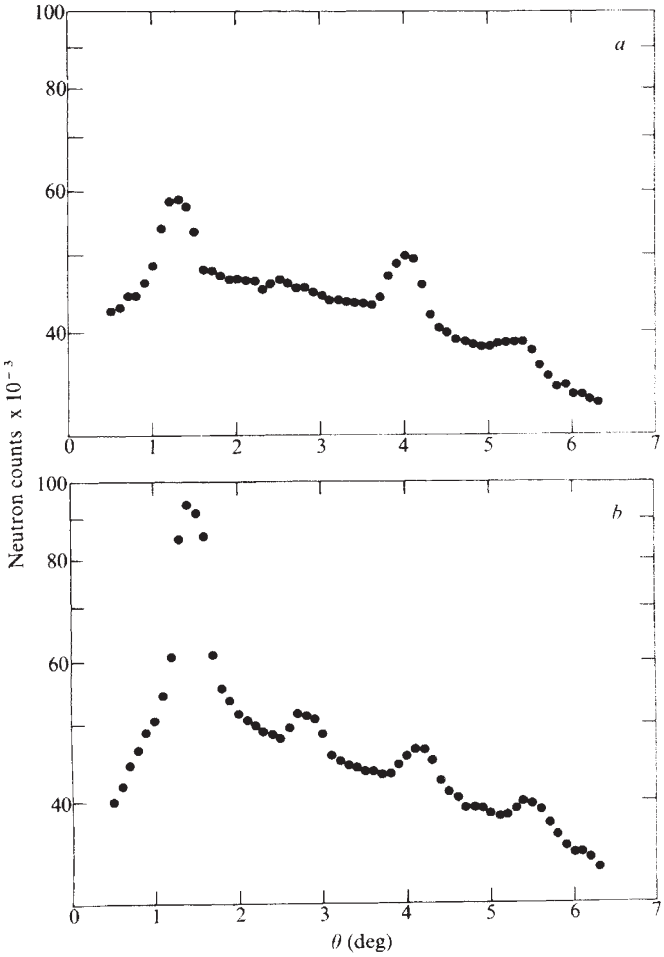

Fig. 1 Diffraction patterns collected at the High Flux Beam Reactor at Brookhaven National Laboratory of the lamellar reflections of DOL multilayers in the presence of deuterated hexane (*a*) and hydrogenated hexane (*b*). Both patterns show four orders of diffraction ($h = 4$) as recorded by a two-dimensional detector in fixed position as the sample was rotated in 0.1° steps through the Bragg angle with respect to the incident beam. The diffraction patterns were the typical lamellar type with a mosaic spread of 0.1–0.4°. The typical *d*-spacing was 48 Å. The sample was oriented on a quartz slide sealed in an aluminium chamber (temperature 22.5 °C). RH was maintained at 66% with a saturated sodium nitrite solution (100% H_2O, 0% D_2O). The vapour pressure (P) of hexane was fixed at 40 mm Hg with a hexane/hexadecane mixture. $P = X_H^m P_0$ where X_H^m is the mole fraction of hexane in the mixture (0.3 in this case) and P_0 is the vapour pressure of pure hexane (134 mm Hg, 22.5 °C). The sample came into equilibrium with the hexane vapour within 2 h as shown by unchanging diffraction patterns.

† To whom correspondence should be addressed (at the University of California).
§ Present address: Solid Photography, Inc., Melville, New York 11746, USA.

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