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W. ZYCH, K. DOŁOWY*, J. OSTROWSKI

CHANGES IN THE MEMBRANE POTENTIAL CAN AFFECT PEPSINOGEN SECRETION OF ISOLATED RAT CHIEF CELLS

Department of Gastroenterology and *Department of Biophysics and Biomathematics, Medical Center of Postgraduate Education, Warszawa, Poland.

The aim of this study was to determine pepsinogen secretion by isolated rat chief cells in relation to changes of membrane potential obtained by the use of potassium ionophore valinomycin and different extracellular K^+ concentrations, or protonophore carbonyl cyanide m-chlorophenyl hydrazone (CCCP) at different pH. While valinomycin or CCCP decreased basal and cAMP-stimulated secretory response of the chief cells, they increased secretion of pepsinogen stimulated by calcium ionophore A23187. When Ca^{2+} was absent from the incubation media, A23187-stimulated secretion of pepsinogen significantly decreased. Moreover, the omission extracellular calcium reversed the potentiating effects of valinomycin or CCCP on A23187-stimulated secretory response.
It is known that pepsinogen secretion is a biphasic process. In the first phase, Ca^{2+} is released from its intracellular stores, while the second one depends on extracellular calcium entering the cell. Our results suggest that this phase is dependent also on membrane potential.

Key words: *gastric glands; pepsinogen; valinomycin; CCCP; calcium ionophore A23187; membrane potential.*

Pepsinogen is secreted by the chief cell which is a representative exocrine cell. It is stored in apical zymogen granules. New molecules of pepsinogen are synthesized in a profuse basal endoplasmic reticulum. The cellular mechanisms controlling secretion of pepsinogen are not understood (1, 2).

In a recently published paper we described a working hypothesis relating tubulovesicle membrane recycling to changes of parietal cell membrane potential (3). If membrane potential played the same role in the fusion of tubulovesicles with secretory canaliculi, a similar effect would be expected in the secretory response of chief cells.

The aim of this study was to compare pepsinogen secretion of isolated rat chief cells in relation to the changes of membrane potential obtained by use of potassium ionophore valinomycin and protonophore carbonyl cyanide m-chlorophenyl hydrazone (CCCP).

MATERIAL AND METHODS

Gland preparation

The gastric glands were isolated according to the method of Gespach (4) described in detail previously (5). After isolation, glands were harvested by centrifugation, washed and resuspended in the incubation medium containing (in mM): 0.5 NaH_2PO_4, 1.0 Na_2HPO_4, 20 $NaHCO_3$, 70 NaCl, 5 KCl, 11 glucose, 1.0 $CaCl_2$, 1.5 $MgCl_2$, 50 HEPES-NaOH, pH = 7.4, and 1 mg bovine albumin/ml. The cell viability of isolated glands, determined by exclusion of 0.4% trypan blue, was over 90% in used preparations.

Measurement of pepsinogen release

After a 30 minute preincubtion at 37° C in incubation medium gassed with 95% O_2 — 5% Co_2 glands were resuspended in the appropriate media. These media were the same as standard incubation medium except that each had a different pH for use in experiments with CCCP. In experiments with valinomycin, potassium ions isotonically replaced sodium ions to a final concentration of 75 mM. Replaced media containing 0.1 mM IBMX were previously gassed for 5 minutes with 95% O_2 — 5% CO_2. A final gland suspensions contained about 10 mg wet wt glands/ml. Next, gland suspensions were incubated in cappped Eppendorf tubes in a shaking water bath for 45 minutes at 37°C. Test agents were added and time O samples were taken at the start of incubation. For determination of total pepsinogen, an aliquot of gland suspension was either lysed in the presence of 1% Triton X-100 or sonicated. Complete cellular disruption was confirmed by light microscope examination. After incubation, an aliquot of gland suspension was centrifuged (10.000 g, 10 sec.), and the supernatant was separated from the pellet. Pepsinogen secretion of chief cells was measured to determine peptic activity in the supernatant, and calculated as the precentage of peptic activity of pepsinogen content in chef cells at the time O.

Peptic activity of pepsinogen was assayed using acidic bovine serum albumin (fraction V) at pH = 2.0 as the substrate accordingly to the method described previously (6).

Statistical analysis

Results are presented as means ± SD. Significant differences between the values were assessed by means of the Student's t test. A p value of < 0.05 was considered significant.

RESULTS

Fig. 1 presents results of the measurement of the effect of potassium ion concentration on the basal and dbcAMP- or A23187-stimulated pepsinogen secretion. In unstimulated cells the effect of high extracellular potassium levels on pepsinogen secretion was not significant, but the secretory response of chief cells stimulated by dbcAMP or A23187 significantly lower in the presence of 75 mM K^+ than in the standard medium containing 5 mM K^+.

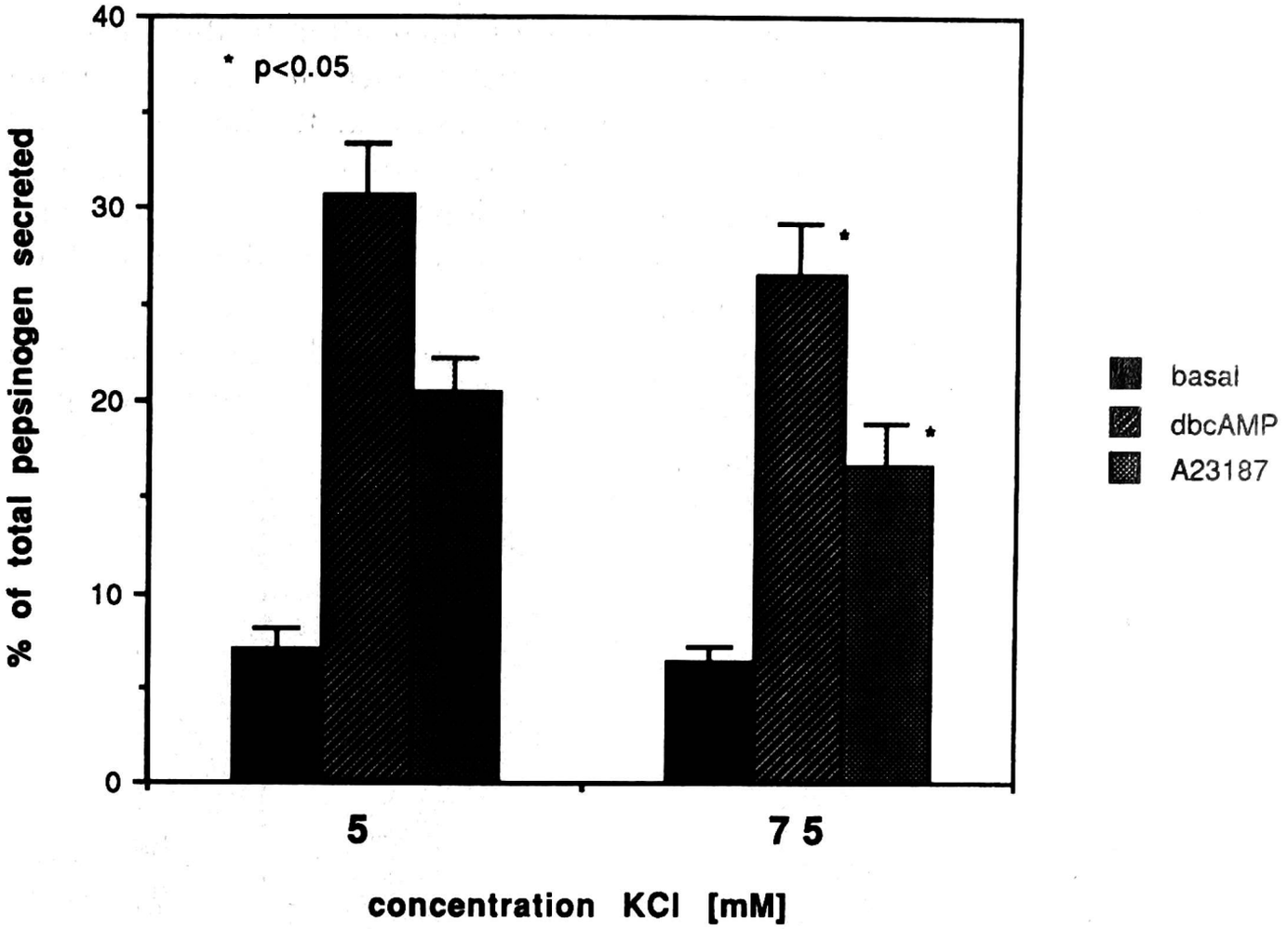

Fig. 1 Graph showing effects of extracellular K^+ concentrations on basal and dbcAMP or A23187-stimulated pepsinogen secretion. Concentrations of dbcAMP and A23187 were 10^{-4}M and 10^{-5}M, respectively. Results are expressed as the percentage of total pepsinogen in the gland suspension that was released into the incubation medium during a 45-min incubation. Results represent mean ± SD of 6 experiments performed on different cell preparations.
* indicates significant decrease ($p < 0.05$) of pepsinogen secretion by dbcAMP or A23187-treated glands in the presence of 75 mM KCl compared to those stimulated in standard medium.

As shown in *Fig. 2,* valinomycin affected the basal and stimulated secretion of pepsinogen. In the presence of 5 mM K^+, valinomycin significantly decreased basal and dbcAMP-stimulated secretion and substantially increased secretion stimulated by calcium ionophore A23187. In the presence of 75 mM extracellular potassium concentration, valinomycin treatment revealed similar effects, but to a significantly greater extent in unstimulated or dbcAMP-stimulated cells, and to a significatly lesser extent in A23187-stimulated cells. Changes of the pepsinogen secretory response to valinomycin treatment in the presence of 140 mM K^+ were similar to those found in glands incubated with 75 mM K^+ (data not shown).

Fig. 3 presents the effect of changes in pH of the incubation medium on the basal and stimulatd secretory response. Pepsinogen secretion was the lowest at the lowest pH, and progressively increased at increasing pH of the medium.

Changes in the basal and stimulated pepsinogen secretion in relation to CCCP treatment also depended on the pH of the incubation medium (*Fig. 4*). The differences between pepsinogen secretion determined in the absence and presence of the protonophore CCCP were the highest at the lowest pH and decreased progressively at increasing pH of the medium. CCCP decreased basal and dbcAMP-stimulated pepsinogen secretion and increased that stimulated by A23187. The effects of CCCP treatment were very similar to those described after valinomycin treatment.

Whereas both valinomycin and CCCP decreased the basal and dbcAMP-stimulated secretory response of the chief cells, they increased pep-

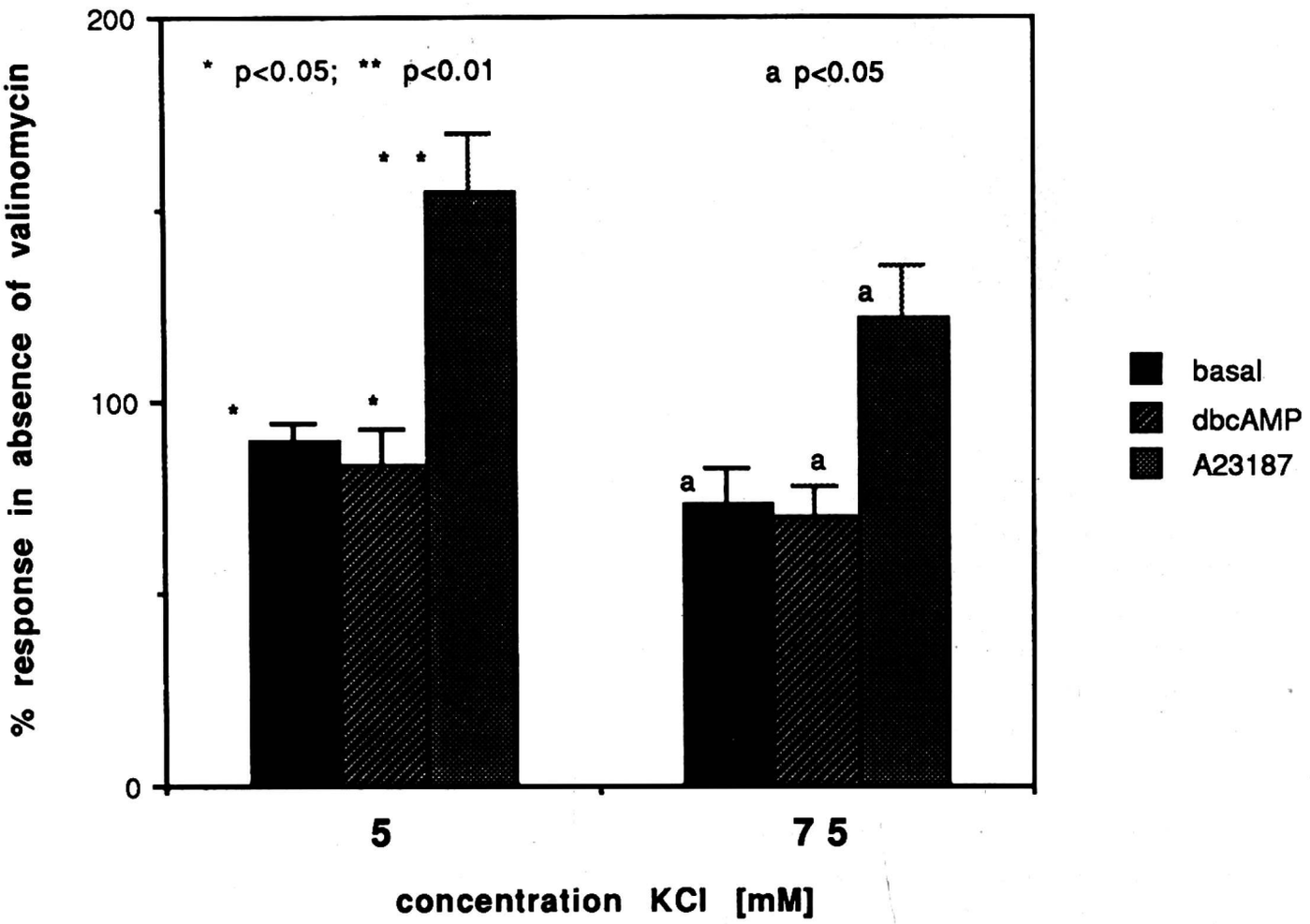

Fig. 2. Graph showing inhibitory effects of 10^{-5}M valinomycin on basal and dbcAMP-stimulated pepsinogen secretion and stimulatory effect of valinomycin on pepsinogen secretory response to A23187 as a function of the extracellular potassium concentration. Concentrations of dbcAMP and A23187 were 10^{-4}M and 10^{-5}M, respectively. Results are expressed as the percentage of pepsinogen secretion observed in the absence of valinomycin at varying K^+ concentrations, as indicated. Results represent mean ± SD of 6 experiments performed on different cell preparations. * indicates significant decrease of pepsinogen secretion by valinomycin- and dbcAMP + valinomycin-treated glands and ** indicates significant increase of pepsinogen secretion by A23187 + valinomycin-treated glands compared to those found in the absence of valinomycin; *a* indicates significant decrease of pepsinogen secretion by glands treated with valinomycin and valinomycin + dbcAMP or A23187 and incubated in 75 mM KCl-medium compared to those treated in the standard medium.

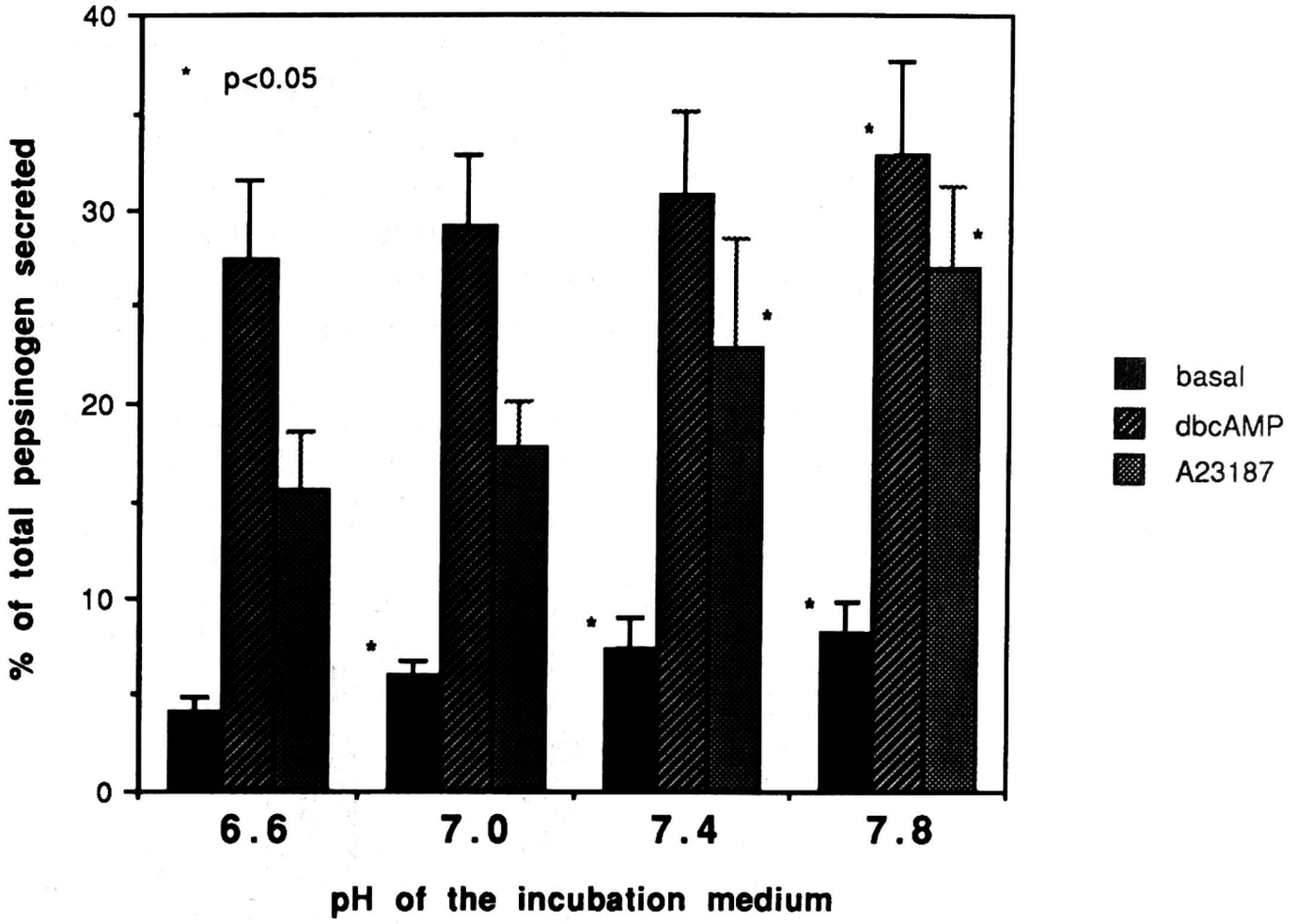

Fig. 3. Graph showing the effect of pH of the incubation medium on basal and dbcAMP or A23187-stimulated pepsinogen secretion. Concentrations of dbcAMPand A23187 were 10^{-4}M and 10^{-5}M, respectively. Results are expressed as the percentage of total pepsinogen in the gland suspension that was released into the incubation medium during a 45-min. incubation. Results represent mean $\pm$ SD of 6 experiments performed on different cell preparations. * indicates significant increase ($p < 0.05$) of pepsinogen secretion by untreated and dbcAMP or A23187-treated glands incubated in media of different pH compared to those incubated in pH = 6.8 of the medium.

sinogen secretion stimulated by calcium ionophore A23187. To examine the role of extracellular calcium ions on the potentiating effects of valinomycin or CCCP on chief cell secretory response to A23187, we used incubation media of two different potasium concentrations (*Fig. 5*) or two different pH (*Fig. 6*) in the presence or absence of calcium ions.

When Ca^{2+} was absent from the incubation media, A23187-stimulated pepsinogen secretion was decreased almost to the basal levels. Moreover, the omission of extracellular calcium reversed the potentiating effects of valinomycin or CCCP on A23187-stimulated secretory response causing the decrease in the secretion of pepsinogen. Thus, these potentiating effects were strongly connected with the calcium inflow into chief cells.

DISCUSSION

As described recently for studying the cellular mechanisms of acid production, cell isolation from rat stomachs without any enzyme digestion seems to be superior to cell preparation by pronase-EDTA method (5). Our experiments performed on gland preparations obtained by the use of hyperosmolar NaCl-EDTA solution revealed sufficient cellular pepsinogen content with the low rate of spontaneous pepsinogen release and adequate responsiveness to stimuli, according to the criteria identified for the study of zymogen secretion by Koelz et al. (7).

External signals detected by surface receptors on the chief cell are transmitted across the plasma membrane, as in other cells, by mechanisms utilizing

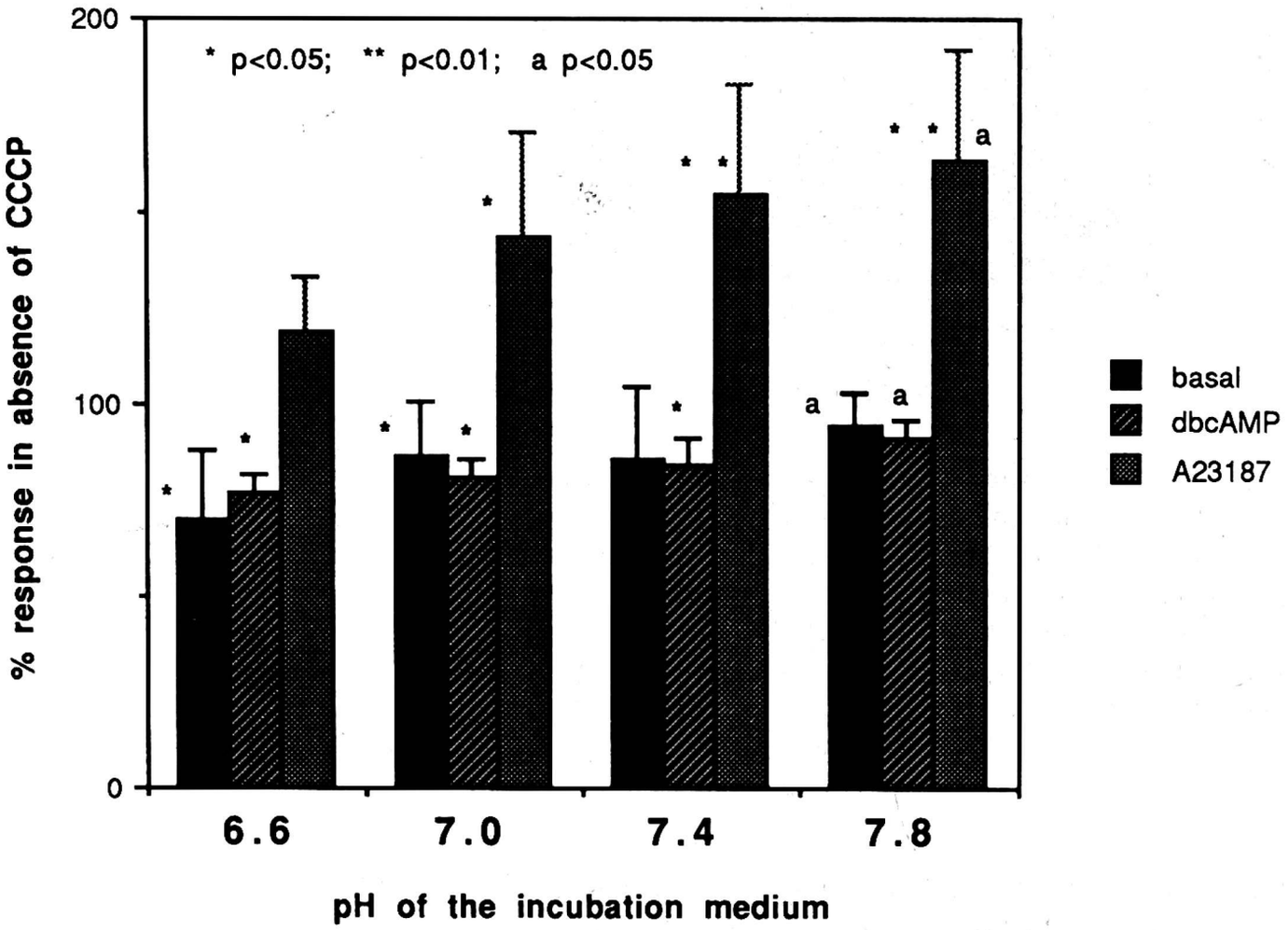

Fig. 4. Graph showing the inhibitory effects of 10^{-6}M CCCP on basal and dbcAMP-stimulated pepsinogen secretion and the stimulatory efect of CCCP on the pepspinogen secretory response to A23187 as a function of pH of the incubation medium. Concentrations of dbcAMP and A23187 were 10^{-4}M and 10^{-5}M, respectively. Results are expressed as the percentage of pepsinogen secretion observed in the absence of CCCP at varying pH as indicated. Results represent mean ± SD of 6 experiments performed on different cell preparation. * indicates significant decrease of pepsinogen secretion by CCCP- and dbcAMP + CCCP-treated glands, and ** indicates significant increase of pepsinogen secretion by A23187 + CCCP-treated glands compared to those found in absence of CCCP; *a* indicates significant increase of pepsinogen secretion by glands treated with CCCP and CCCP + dbcAMP or A23187 and incubated in pH = 7.8 of the medium compared to those treated in pH = 6.6.

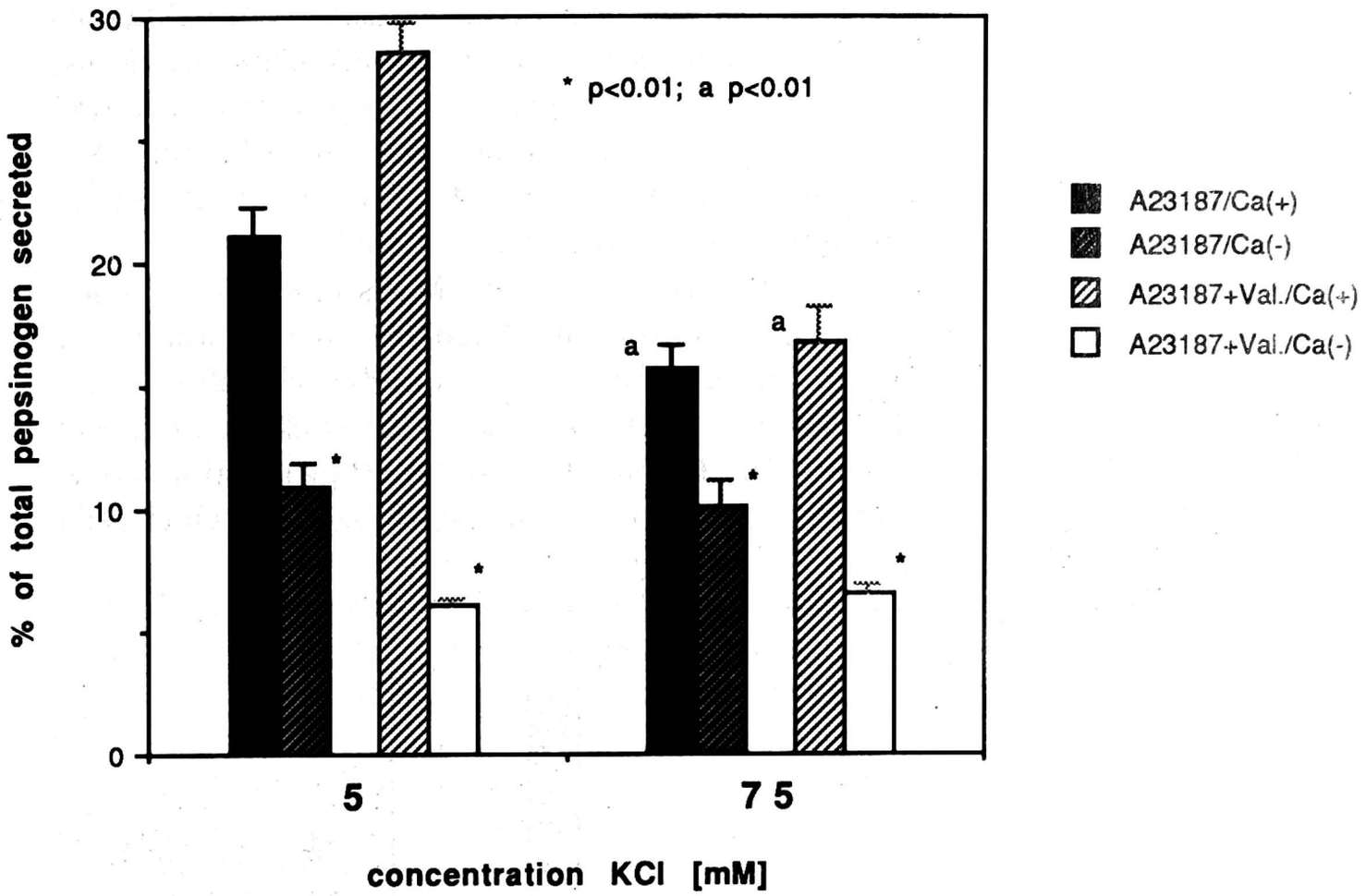

Fig. 5. Graph showing effects of 10^{-5} valinomycin and extracellular calcium removal on changes in basal and A23187-stimulated pepsinogen secretion as a function of extracellular K^+ concentrations. Concentration of A23187 was 10^{-4}M. Results are expressed as the percentage of total pepsinogen in the gland suspension that was released into the incubation medium during a 45-min. incubation. Results represent mean ± SD of 3 experiments performed on different cell preparations. * indicates significant decrease of pepsinogen secretion by A23187- or A23187 + valinomycin-treated glands in the absence of calcium ions compared to those treated in the presence of Ca^{2+} in both 5 and 75 mM KCl-media; a indicates significant decrease of pepsinogen secretion by A23187- or A23187 + valinomycin-treated glands in the presence of 75 mM KCl compared to those in the presence of 5 mM KCl.

cyclic nucleotides, inositol lipid derivatives, and calcium ions. Pepsinogen secretion by chief cells is modulated by both cAMP-dependent and calcium-dependent pathways (8—12). Our experiments were performed on glands stimulated by 10^{-}M dbcAMP or 10^{-5}M calcium ionophore A23187. Whereas dbcAMP directly activates the cAMP-dependent system, A23187 promotes calcium ion transport across biological membranes which transiently elevates concentration of Ca^{2+} in the cytoplasm, activating of the calcium-dependent system.

Valinomycin, a potassium-specific carrier, is able to establish a cell membrane potential that depends on K^+ concentration of the incubation medium. Cell membrane potential can also be changed by proton gradients in the

presence of protonophore, e.g. CCCP which passively transports H^+ down its electrochemical gradient, producing a membrane potential according to Nernst equation for protons (14—16). In described experiments pepsinogen secretion was determined in the presence of 10^{-5}M valinomycin and different K^+ concentrations of the incubation media or 10^{-6}M CCCP in standard incubation medium at four different pH levels: 6.6, 7.0, 7.4, and 7.8.

Valinomycin and CCCP decreased basal and dbcAMP-stimulated secretion of pepsinogen and increased the secretion stimulated by calcium ionophore A23187. The decreasing effect on pepsinogen secretion by ionophores was likely due to an important side effect of them, which is a blockade of energy production in mitochondria (14—16). This inhibition of ATP production would interfere with the production and/or secretion of pepsinogen by chief cells.

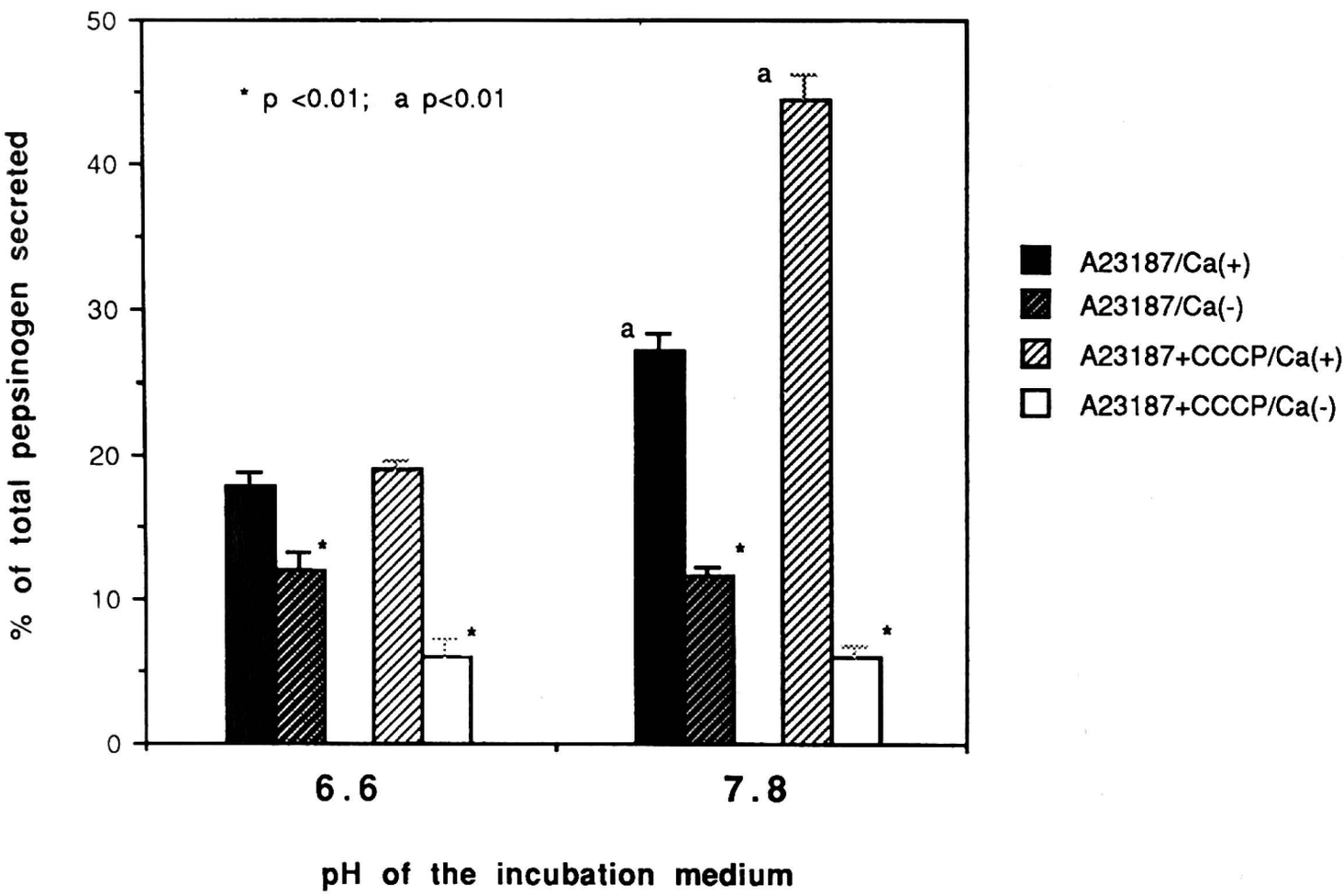

Fig. 6. Graph showing the effects of 10^{-6}M CCCP and extracellular calcium removal on changes in basal and A2317-stimulated pepsinogen secretion as a function of pH of the incubation medium. Concentration of A23187 was 10^{-5}M. Results are expressed as the percentage of total pepsinogen in the gland suspension that was released into the incubation medium during a 45-min incubation. Results represent mean ± SD of 3 experiments performed on different cell preparation. * indicates significant decrease of pepsinogen secretion by A23187- or A23187 + valinomycin-treated glands in the absence of calcium ions compared to those treated in the presence of Ca^{2+} in both pH = 6.6 and pH = 7.8 of the incubation media; a indicates significant increase of pepsinogen secretion by A23187- or A23187 + valinomycin-treated glands in pH = 7.8 of the incubation medium compared to those treated in pH = 6.6.

The predominantly mitochondrial role in decreasing the chief cell secretory response by valinomycin or CCCP was overcome by high extracellular K^+ and higher pH of the incubation medium.

It is known that pepsinogen secretion, activated by cholinergic or peptidergic receptors, is a biphasic process (17—20). In the first phase, pepsinogen secretion is mediated by calcium ions released from the calcium intracellular stores, while the second phase depends on the extracellular calcium entering the cell.

The key results, summarizing the effect of A23187 stimulation on the secretion of pepsinogen, are shown in *Fig. 5* and *6*: *Firstly*, pepsinogen secretion was much higher with calcium in the incubation medium than in its absence. However, the presence of calcium in the medium is not a prerequisite to secretion, i.e. the first phase of secretion is dependent on internal calcium stores. *Secondly*, with calcium in the incubation medium pepsinogen secretion was considerably higher if the membrane was hyperpolarized (low potassium plus valinomycin or high pH plus protonophore), and remained unchanged in cells treated with valinomycin plus high potassium concentration or protonophore at low pH. *Thirdly*, valinomycin or CCCP decreased the ability of chief cells to secrete pepsinogen, and the inhibitory effect of these ionophores was reversed by increased intracellular Ca^{2+} levels by A23187 treatment. When calcium was absent in the incubation medium, both protonophore and valinomycin inhibited pepsinogen secretion, even in the presence of A23187.

From these results we conclude that the second phase of calcium-dependent secretory response of chief cells, caused by extracellular calcium inflow into the cell, depends on membrane potential.

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Author's address: J. Ostrowski, Department of Gastroenterology, 02-616 Warszawa, Goszczyńskiego 1, Poland.