different volumetric and light-scattering changes that lead to the suggestion that Cl^- can penetrate through the membranes whereas acetate, propionate, formate and phosphate, not only penetrate through the membranes but also penetrate 'into' the membranes, triggering conformational changes in this structure[9]. Therefore it is possible that acetate and other releated ions act in a similar manner upon chromaffin granule membranes and modify the final response to ATP.

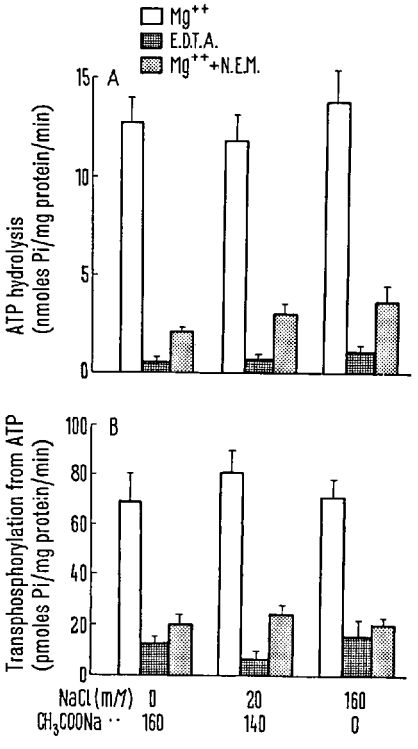

Fig. 3. Effect of chloride and acetate ions on Mg^{++}-dependent ATPase activity and transphosphorylation of Pi from ATP to chromaffin granule membranes. Chromaffin granules were incubated for 20 min at 30 °C in media containing 3 different concentrations of Cl^- and CH_3COO^-. The incubation media contained 0.5 m*M* Mg^{++} and 0.5 m*M* [γ-^{32}P] ATP (specific activity, 0.54 μCi/μmole). When EDTA was present, Mg^{++} was omitted from the incubation medium. EDTA (2.0 m*M*) or N-ethyl-maleimide (0.2 m*M*) was added to the medium 5 min prior to the addition of [γ-^{32}P]ATP. Addition of 10% trichloroacetic acid terminated the reactions. ATPase activity (A) and transphosphorylation of Pi from ATP (B) were determined as indicated in methods. Each bar represents the mean $\pm$ S.E. of 4 separate experiments.

Under our experimental conditions, 20 m*M* of Cl^- was necessary for the ATP-evoked release reaction. The intracellular concentrations of Cl^- has not yet been determined in chromaffin cells, but if we assume a passive distribution of Cl^- according with the transmembrane potential[10], and using the known experimental values for the resting potential (–24 to –30 mV) of chromaffin cells[11], the calculated (theoretical) intracellular concentration of Cl^- should be between 38 and 47 m*M*. This concentration is greater than that necessary to support ATP-evoked catecholamine release in vitro. However, preliminary experiments with bovine adrenal glands perfused with Locke's solution containing either sodium chloride, sodium acetate or sodium sulfate showed no differences when catecholamine release was evoked by 56 m*M* of KCl or CH_3COOK in the presence of 2.2 m*M* Ca^{++}, but at the end of the 2 h perfusion period the tissue level of Cl^- in the medullae was 19 m*M* (14–24, $n = 5$) as determined by the method of MATUK et al.[12]. Further studies using radioactive traces will be necessary before definitive conclusions about the role of Cl^- in the secretory process can be made.

Résumé. L'ATP induit la libération de catécholamines des granules chromaffines médullaires. Nous discutons le rôle du Cl^- dans ce processus vis-à-vis l'activité de l'ATPase et la phosphorylation des membranes. Nos résultats indiquent que la réaction aboutissant à la liberation de catécholamines est un phenomène comportant plusieurs étapes échelonnées après l'hydrolyse de l'ATP.

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The Activation of Rat Stomach Histidine Decarboxylase is Independent of the Histamine Level

Vagal excitation (insulin hypoglycemia), feeding or injection of gastrin are known to mobilize gastric mucosal histamine and to activate gastric histidine decarboxylase in the fasted rat[1,2]. Recently, it was suggested that the histamine-mobilizing and enzyme-activating effects of vagal excitation and feeding are mediated by endogenous gastrin[3–5]. KAHLSON et al.[1,2] have proposed that mobilized histamine stimulates the parietal cell to secrete and that histamine is the final common mediator of the acid-stimulating effect of both gastrin and vagal excitation. From the observations that the gastrin-induced reduction of gastric histamine preceded the activation of the histamine-forming enzyme, and that exogenous histamine reduced the enzyme activity, it was concluded that the histidine decarboxylase activity is dependent upon the mucosal histamine content via feed-back mechanism: a low level of gastric histamine induces enzyme synthesis to replenish the mucosal histamine stores, a high level of gastric histamine has a repressive effect on enzyme synthesis[1,2]. However, certain experimental observations are at variance with the above concept. At a certain dose level, insulin acti-

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vates gastric histidine decarboxylase without affecting the gastric histamine content[6]. Porta-caval-shunted rats have an increased gastric histamine content as well as mark edly increased enzyme activity[7,8]. Vagal denervation causes a dramatic increase in the gastric histidine decarboxylase activity, although the level of gastric histamine remains normal[4,9,10]. Finally, removal of the endogenous gastrin stores by antrectomy reduces both gastric histamine and gastric histidine decarboxylase activity[3,11]. The latter finding suggests that low gastric histamine in itself does not activate histidine decarboxylase. Rather, it seems that gastrin exerts a dual action on the gastric histamine-storing cell in that it mobilizes histamine, concomitantly – but independently – stimulating the synthesis of histidine decarboxylase. This assumption was tested by following the changes in the gastric histamine content and the gastric histidine decarboxylase activity after repeated injections of pentagastrin, and by comparing the histamine content and the enzyme activity in fasted, re-fed and freely fed rats.

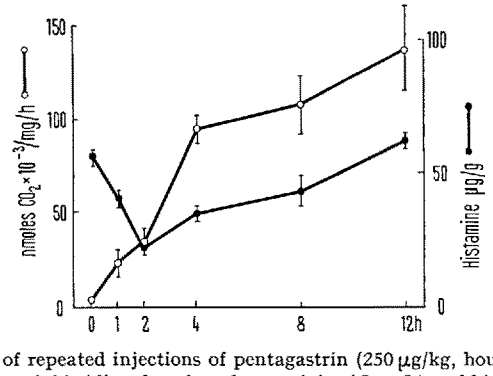
Male Wistar rats (weighing 150–250 g) were fed a diet of food pellets ad libitum or fasted for 48 h (free access to water) before sacrifice. One group of fasted rats was given access to food for 2 h before sacrifice. Another group received pentagastrin (Peptavlon®, 250 µg/kg, s.c.) hourly for various times up to 12 h and were killed after 1, 2, 4, 8 and 12 h by exsanguination under light ether anaesthesia. Fasted controls were given 0.9% saline and killed 1 or 2 h later or sacrificed without any treatment. The mucosa of the oxyntic gland area was scraped off, weighed and homogenized in 0.1 M phosphate buffer, pH 6.9, to a final concentration of 100 mg tissue (wet weight) per ml. After centrifugation at 5,000 $\times g$ for 10 min in a refrigerated centrifuge, aliquots (0.4 ml) of the supernatant were taken for assay of histidine decarboxylase[4] using 1-^{14}C-L-histidine (specific radioactivity: 1.3 mc/mM; New England Nuclear) as substrate. Composition of incubation mixture: Gastric mucosal extract (corresponding to 40 mg mucosa, wet weight), 1-^{14}C-L-histidine ($4 \times 10^{-4} M$), pyridoxal-5'-phosphate ($10^{-5} M$) and glutathione ($5 \times 10^{-4} M$) in a total volume of 0.5 ml were incubated under nitrogen at 37 °C for 1 h. Enzyme activities were expressed as nmoles $^{14}CO_2$ produced per mg tissue per hour. All assays were run in duplicate. For determination of gastric histamine the proteins of the mucosal homogenate were precipitated with 5% trichloroacetic acid. The deproteinized samples were extracted at an alkaline pH with a mixture of n-butanol and chloroform (3:1) as previously described[12]. Extraced histamine was determined fluorometrically[12,13].

The level of gastric histamine in freely fed rats was lower ($0.01 < P < 0.05$) than in fasted rats but higher ($P < 0.001$) than in fasted, refed rats. Refeeding caused a significant elevation of the gastric histidine decarboxylase activity, but the enzyme activity was higher ($0.001 < P < 0.01$) in freely fed rats (Table).

1 h after a single injection of pentagastrin, the gastric histamine of the fasted rats was markedly reduced ($0.001 < P < 0.01$) and the gastric histidine decarboxylase activity was moderately increased ($0.01 < P < 0.05$); injection of saline was without effect (Table). Repeated injections of pentagastrin caused a progessive increase in the enzyme activity. The histamine content was maximally reduced after 2 injections of pentagastrin, thereafter it increased, reaching normal values after 12 h (Figure). According to the concept of KAHLSON et al.[1,2], the activation of gastric histidine decarboxylase is a consequence of the gastrin-induced reduction of mucosal histamine. Since repeated injections of gastrin (and pentagastrin) cause a progressive increase in the enzyme activity[1], this hypothesis presupposes a progressive reduction of the gastric histamine content, or at least a persistently low level of gastric histamine. In the present study, it could be shown that after an initial reduction, gastric histamine reaches normal levels after about 12 h of pentagastrin treatment, conceivably as a result of an accelerated rate of histamine formation. It may therefore be concluded that the gastrin-induced activation of gastric histidine decarboxylase is not dependent upon a reduced gastric histamine level[14].

Histidine decarboxylase activity and histamine content in gastric mucosa. Effect of fasting, feeding and pentagastrin treatment. Means ± SEM (n).

	nmoles $CO_2 \times 10^{-3}$/mg/h		µg histamine/g	
Freely fed	47.0 ± 6.3	(10)	40.0 ± 5.9	(10)
Fasted (48 h)	5.0 ± 0.7	(10)	55.6 ± 2.8	(13)
Fasted, refed	24.2 ± 2.5	(18)	30.4 ± 4.2	(9)
Fasted, pentagastrin 250 µg/kg, s.c.	23.8 ± 6.7	(6)	41.1 ± 3.2	(15)
Fasted, saline 0.9% 10 ml/kg, s.c.	3.8 ± 1.4	(7)	62.5 ± 6.7	(8)



Effect of repeated injections of pentagastrin (250 µg/kg, hourly) on rat stomach histidine decarboxylase activity (○—○) and histamine content (●—●). Means ± S.E.M., at least 5 animals in each group.

Zusammenfassung. Nach wiederholter Pentagastrin-Injektion wurde ein progressiver Anstieg der gastrischen Histidin-Decarboxylase-Aktivität in nüchternen Ratten gefunden. Nach anfänglicher Abnahme steigt der gastrische Histaminspiegel und erreicht nach 12 stündiger Behandlung Normalwerte, was beweist, dass die pentagastrininduzierte Aktivierung der Enzymaktivität vom Histaminspiegel unabhängig ist.

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