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1 Acknowledgments. The author gratefully acknowledges the excellent technical assistance of I. Bolliger, J. Heller, S. Küng, B. Müller, B. Schmidt and U. Völkle.
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0014-4754/84/050476-03$1.50 + 0.20/0

Potentiating action of hexoprenaline on ^{14}C-aminopyrine uptake by isolated rat parietal cells

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26 June 1983

Summary. Hexoprenaline potentiated the ^{14}C-aminopyrine uptake (a reliable index of H^+ generation) of isolated rat gastric cells stimulated by 10^{-6} –10^{-4} mol/l carbachol, and inhibited that in response to 10^{-4} mol/l histamine without and in the presence of propranolol. It is concluded that hexoprenaline acts as a partial agonist on parietal cell H_2-receptors and that β-adrenoceptor activation may functionally modulate gastric acid secretion.

The presence of histamine, acetylcholine and gastrin-receptor sites on parietal cells is well documented[2]. Secretagogues interact and potentiate each other in vivo[3] and in vitro[4]. Histamine exerts its action by stimulation of H_2-receptor sensitive adenylate cyclase which then initiates the intracellular steps of H^+ production[5]. It was previously shown that β-adrenergic stimulation also activates gastric mucosal adenylate cyclase[6-9]. Moreover, we found that the selective β_2-receptor agonist hexoprenaline stimulated the ^{14}C-aminopyrine (^{14}C-AP) uptake of isolated gastric cells[9], a reaction which reflects acid production inside the parietal cell[10]. The present investigation studied the interaction of hexoprenaline on the ^{14}C-AP uptake in response to histamine or cholinergic stimulation by carbachol.

Experimental. Cell isolation and ^{14}C-AP uptake measurements have been described previously in detail[9,11]. All experiments with histamine were performed in the presence of 10^{-3} mol/l isobutylmehtylxanthine (IBMX) and 10^{-3} mol/l Ca^{2+}. The carbachol experiments were done without IBMX and in the presence of 2×10^{-3} mol/l Ca^{2+}. The results were evaluated by the t-test for paired data.

Results. The effect of hexoprenaline, alone and with 10^{-4} mol/l histamine, a maximal effective concentration, on ^{14}C-AP uptake is shown in figure 1. Hexoprenaline stimulated the basal uptake by 100% ($p < 0.001$), 10^{-5} mol/l being the maximal effective concentration[9]. The adrenergic β-receptor antagonist propranolol reduced the hexoprenaline stimulation and 10^{-7} mol/l were almost sufficient to abolish any response. The histamine H_2-receptor antagonist cimetidine also exerted inhibitory potencies; however, concentrations higher than 10^{-7} mol/l were required.

The histamine-stimulated ^{14}C-AP uptake was much more pronounced than that following hexoprenaline, and was significantly reduced by 16.5–30.5% in the presence of 10^{-6}–10^{-4} mol/l hexoprenaline. Propranolol (10^{-7} mol/l) reduced the histamine stimulation by 12% (n.s.), but did not reverse the action of 10^{-4} mol/l hexoprenaline, which decreased the histamine effect by 38% ($p < 0.01$) in the presence of the adrenergic β-blocker. When lower, i.e., threshold concentrations of histamine (10^{-6} mol/l) were used, the results were less clear and in some experiments even potentiation of ^{14}C-AP uptake was observed.

For optimal cholinergic stimulation the cell medium had to be IBMX free and contained 2×10^{-3} mol/l Ca^{2+}. Carbachol stimulated the AP uptake in a concentration-dependent manner, maximally by 85% (10^{-4} mol/l). Without IBMX, hexoprenaline alone evoked no response. However, the β_2-receptor agonist increased significantly the ^{14}C-AP uptake due to carbachol, which now stimulated the uptake maximally by 134%. It seems that this effect represents true potentiation, since the values are higher than the calculated sum of responses to both compounds.

Discussion. The data confirm and extend our previous findings with β-receptor agonists[9] and demonstrate a potentiating interaction between hexoprenaline and carbachol on ^{14}C-AP accumulation of isolated rat gastric cells. They are partly contradictory to the general view that adrenergic stimulation solely inhi-

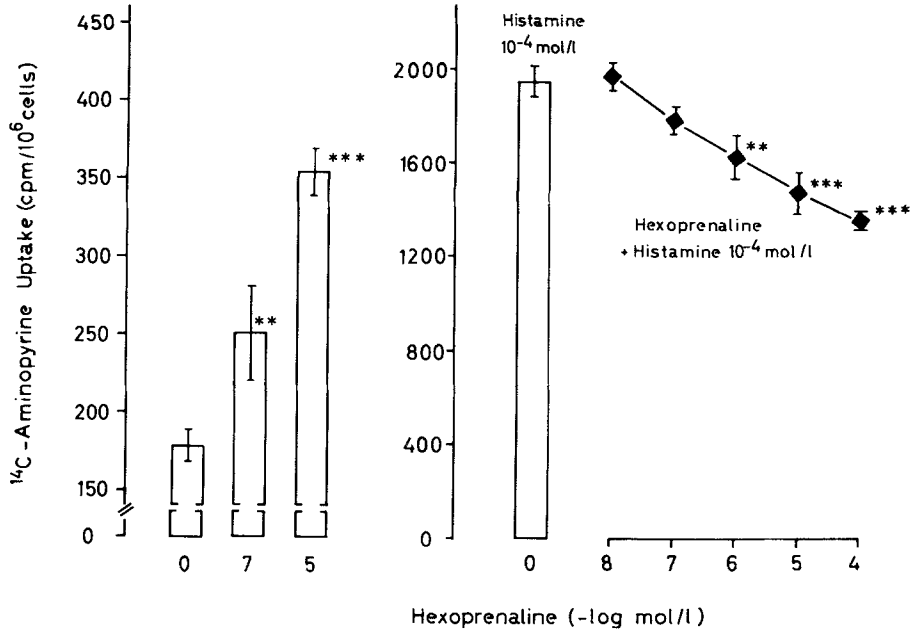

Figure 1. Influence of hexoprenaline on the histamine-stimulated ^{14}C-aminopyrine uptake of isolated gastric mucosal cells with 25 ± 2.2% parietal cells. Left: Effect of hexoprenaline (10^{-7} and 10^{-5} mol/l) alone. Right: Effect of hexoprenaline (10^{-8} to 10^{-4} mol/l) on the histamine (10^{-4} mol/l) stimulated ^{14}C-AP uptake. All experiments were carried out in the presence of IBMX and Ca^{2+} at a concentration of 10^{-3} mol/l. Values $\bar{x}$ ± SEM, N = 5, **p < 0.01, ***p < 0.001.

bits gastric acid secretion[12], and support recent experiments with isolated rat stomach preparations responding to β_2-adrenoceptor stimulation by acid production[13, 14]. Some previously published in vivo studies have also indicated that β-receptor agonists increase[15] and β-receptor antagonists decrease acid secretion[16] in rats. It was suggested that β-receptor agonists exert a certain intrinsic activity in relation to H^+ production initiated by activation of adenylate cyclase and blocked by propranolol[9]. It seems that this effect can only be seen in the absence of physiological regulatory mechanisms, using isolated cells or an isolated stomach preparation[9, 13, 14].

In vivo experiments are difficult to interpret, because of the broad spectrum of catecholamine effects with dominating haemodynamic and neurohormonal interactions. Nevertheless, most in vivo experiments have shown that β-adrenergic agonists inhibit gastric acid secretion (for review of the numerous publications see Burnstock and Wong[12]) and that histamine-induced gastric secretion is more difficult to suppress with catecholamines than that in response to pentagastrin[17]. This peptide was not studied, since pentagastrin does not stimulate ^{14}C-AP uptake of rat gastric cells.

Curwain et al.[18] and Lundell and Svensson[19] showed an increase in H^+ production and gastric mucosal histamine formation after propranolol treatment. They suggested a causal link between adrenergic and histaminergic systems, the latter under the inhibitory control of catecholamines. Our results showed an increase of ^{14}C-AP uptake caused by hexoprenaline alone, and inhibition of the maximally-stimulated H^+ production in response to histamine *without* and in the presence of an adrenergic β-blocker. They indicate interaction at the receptor level and favor the view that hexoprenaline, like some other adrenergic agents[20], may be a partial agonist of histamine H_2-receptors localized on the parietal cell. Indirect β-adrenoceptor mediated effects cannot be totally excluded, but the experiments in the presence of propranolol do not favor this explanation. The phenomenon that the effect of 2 agents in combination is greater than the sum of the effects of each agent alone is considered as potentiation[3] and this definition is suitable for studies with isolated gastric mucosal cells[8]. Potentiating interactions have been found between histamine and carbachol in isolated

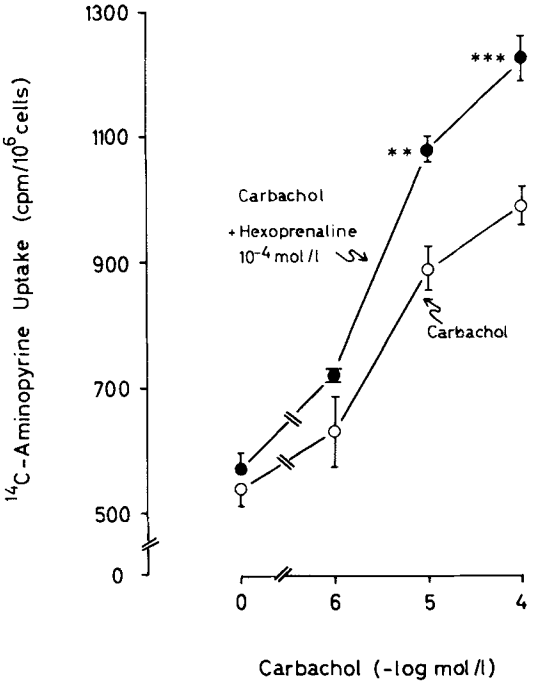

Figure 2. Influence of hexoprenaline (10^{-4} mol/l) on the carbachol (10^{-6}–10^{-4} mol/l) stimulated ^{14}C-AP uptake of isolated gastric mucosal cells with 23.8 ± 1.8% parietal cells. All experiments were carried out without IBMX and in the presence of 2×10^{-3} mol/l Ca^{2+}. Values $\bar{x}$ ± SEM, N = 6, **p < 0.01, ***p < 0.001.

canine[2] and rat[21] parietal cells and between gastrin and histamine in dog and rabbit cell preparations[2,22]. Whether the different mechanisms of action – elevation of endogenous cAMP (histamine) and adenylate cyclase independent transmitter systems (gastrin and carbachol) – are involved in these events is not known. Anyway, according to this view and the concept of hexoprenaline as a partial histamine H_2-receptor agonist, potentiation of ^{14}C-AP uptake due to carbachol is conceivable. The cell preparations used consist of 20–30% of parietal cells. Therefore, we cannot exclude the possibility that hexoprenaline releases gastrin or histamine from endocrine nonparietal cells. The release of gastrin by adrenaline[23], as well as that of histamine by some adrenergic agents, has been reported[20]. In some of our experiments (data not shown), we used cells prepared from the whole rat stomach, including the antrum. In these preparations far more gastrin containing cells are available, but the results were similar. Therefore, it seems reasonable to suspect that release of gastrin does not contribute to potentiation between hexoprenaline and carbachol; however, more studies with enriched fractions of different pools of gastric mucosal cells are necessary to elucidate the mechanisms involved in the action of hexoprenaline on gastric cells. It may be concluded that β-adrenoceptor stimulation functionally modulates gastric acid secretion.

1 Acknowledgments. S. Maśliński, Institute of Rheumatology, Warszawa, held a fellowship of the Alexander v. Humboldt-Foundation. The study was supported by the Deutsche Forschungsgemeinschaft. The skilful technical assistance of Mrs R. Maier and Mr R. Beer is gratefully acknowledged.

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0014-4754/84/050478-02$1.50 + 0.20/0

Changes in hepatic glycosaminoglycans following endotoxin administration

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Summary. Incorporation of [35]S-sulfate into hepatic glycosaminoglycans (GAGs) is affected by intravenous administration of endotoxin. There are significant increases in total labeled glycosaminoglycans and in the percentage of labeled dermatan sulfate 72 h post-endotoxin.

Following intravenous administration of endotoxin, the classical systemic manifestations include an increase in vascular resistance[1,2] and capillary permeability as well as alterations in pulmonary ventilation and fever[3–5]. In addition, endotoxin has been shown to cause necrosis of liver tissue and hemorrhage with the infiltration of leukocytes[6,7]. Although the liver is the primary organ responsible for the detoxification and excretion of endotoxin, hepatocellular damage with decreased bile production occurs following endotoxin administration. Changes in the rate of synthesis and distribution of GAGs have been observed in different organs in response to tissue damage[8–13]. In carbon tetrachloride-induced liver disease, there is an increase in the synthesis of all GAGs and an increase in the relative amount of dermatan sulfate[9,14]. Alterations in the normal distribution of GAGs have also been noted in various forms of hepatic neoplasms[15,16]. It is the purpose of this study to investigate the effect of intravenous endotoxin administration on GAGs in the liver.

Materials and methods. Female Sprague-Dawley rats weighing 190–200 g and fed an unrestricted commercial diet were used throughout. Rats were injected i.v. with 0.5 ml of an endotoxin suspension consisting of 1 mg *E. coli* lipopolysaccharide (Sigma Chemical Co., St. Louis, MO) per 1 ml sterile 0.9% NaCl. They were labeled by i.p. injection of a total dose of 400 µCi [35]S-sulfate (New England Nuclear, Boston, MA), given in 2 parts: 0, 12, 48, and 72 h following endotoxin administration and again 12 h later. This procedure has been used to label glycosaminoglycans and measure their turnover rates both in vivo and in vitro[8,13,17].

Rats were sacrified by i.p. injection of chloral hydrate 12 h