noradrenaline release by exocytosis[16]. Hence, it may be concluded that the selective inhibition of acetylcholine-induced noradrenaline release caused by low pentobarbital concentrations is due neither to an impairment of the exocytotic release mechanism per se, nor to an inhibition of depolarization, nor to a decrease in Ca^{2+} inward current. The latter conclusion implies that Ca^{2+} influx occurs via unspecific Ca^{2+} channels which can be opened by all methods of stimulation used. However, the possibility must be considered that specific Ca^{2+} channels are opened by activation of the nicotinic receptor. This receptor is a highly hydrophobic protein which traverses the lipid matrix of the membrane[17]; both the binding site for acetylcholine and the ionophore involved in the translocation of ions are localized and coordinated within this macromolecule[17]. Evidence has been presented that barbiturates are able to cause a conformational change of membrane proteins[4]. Taken together, we conclude that these drugs may induce a conformational change of the nicotinic receptor which may either block the gating mechanism for the opening of specific Ca^{2+} channels, or prevent the interaction of acetylcholine with the receptor, thus inhibiting stimulus formation. This conclusion is consistent with our finding that pentobarbital causes a non-competitive inhibition of the effect of nicotinic receptor stimulation.

Influence of pentobarbital on the spontaneous noradrenaline output from isolated rabbit hearts and on the removal of exogenous noradrenaline from the perfusion fluid

Pentobarbital concentration (mM)	Noradrenaline output (ng/2 min)*	Removal of noradrenaline (% of the amount infused)**
0	2.8 ± 1.6	41.0 ± 3.5
0.32	3.3 ± 2.3 (n.s.)	47.0 ± 8.2 (n.s.)
1.0	3.9 ± 1.1 (n.s.)	48.4 ± 4.4 (n.s.)

Means ± SEM (N = 5–10). n. s., not significantly different from controls. *Pentobarbital was present in the perfusion fluid 8 min before and during sampling of the perfusates. **Noradrenaline was infused into the aortic cannula for 10 min to give a final concentration of 59 nM. Pentobarbital was present 10 min before and during noradrenaline infusion.

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Effect of carbenoxolone on phosphodiesterase and prostaglandin synthetase activities[1]

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Summary. Carbenoxolone inhibited in vitro cAMP and cGMP phosphodiesterases in a concentration-dependent and noncompetitive manner. Prostaglandin synthetase activity of rabbit kidney medulla was slightly stimulated by carbenoxolone 0.1–0.5 mM, but inhibited by higher concentrations.

Glycyrrhizic acid is one of the numerous substances which have been extracted from liquorice root. Its aglycone is glycyrrhetinic acid, from which carbenoxolone sodium is synthesized. Carbenoxolone has been used in the treatment of gastric and duodenal ulcers[2,3], and it was the first drug convincingly shown to accelerate the rate of healing of chronic gastric ulcer[4]. The mode of action is uncertain, but it seems likely that the drug increases the defensive reactions of the stomach by stimulating or by altering the physical characteristics of mucous secretion[5,6]. The clinical use of carbenoxolone is limited by side-effects due to salt and water retention and potassium loss.

Cyclic adenosine-3′,5′-monophosphate (cAMP) has been suggested to be an intracellular mediator of histamine-induced acid secretion[7], and cyclic guanosine-3′,5′-monophosphate (cGMP) seems to participate in pentagastrin-stimulated acid formation[8,9] Prostaglandins (PGs), on the other hand, have been suggested to function as a physiological brake in the gastric secretion[10]. On this basis, it seemed important to study the effect of carbenoxolone on these agents.

Materials and methods. Phosphodiesterase activities of the fundus part of rat stomach were measured using ^{3}H-cAMP or ^{3}H-cGMP as substrates according to the method of Thompson and Appleman[11]. The inhibitory effects of drugs were measured in duplicate at 5–6 different substrate concentrations (0.1–2.0 μM). K_m, V_{max} and K_i values were calculated from the double reciprocal plot of the Michaelis-Menten equation.

Rabbit kidney medulla has a high PG synthetase activity and is routinely used for evaluating PG formation. In the present study, microsomal fraction of rabbit kidney medulla was used as an enzyme source[12] for measuring the effect of carbenoxolone on PG synthetase. PGE was determined on superfused hamster stomach strip[13].

Drugs and chemicals. ^{3}H-cAMP (27.5 Ci/mmole) and ^{3}H-cGMP (21 Ci/mmole) were supplied by The Radiochemical Centre, Amersham, England. Arachidonic acid (99%) and bovine serum albumin (Sigma Chemicals Co, St. Louis, Mo., USA), hydroquinone (Fluka AG, Buchs, Switzerland), reduced glutathione (E. Merck, Darmstadt, Federal Republic of Germany), carbenoxolone sodium (MS Chemicals, Milano, Italy) and theophylline (pH. Nord.) were used.

Results. Phosphodiesterase. The K_m-values for rat gastric mucosa phosphodiesterase were 1.3 μM for cAMP and 2.0 μM for cGMP. Carbenoxolone concentration-dependently (50–100 μM) inhibited the phosphodiesterases for cAMP and cGMP. The inhibitor constant (K_i) for cGMP phosphodiesterase was 0.22 mM. The type of inhibition was noncompetitive (figure 1). The noncompetitive type of cAMP phosphodiesterase inhibition described by Amer et al.[14] was only once obtained. The inhibitor constant was then 0.042 mM. Other experiments gave the results plotted in figure 1.

Carbenoxolone seemed to activate cAMP degradation at high substrate concentrations. A strong substrate inhibition of cAMP phosphodiesterase was obtained at

substrate concentrations above 5 μM. This inhibition was prevented by carbenoxolone at concentrations of 50 μM. This could explain the complex inhibitory action of carbenoxolone. However, no remarkable bending was obtained in the double reciprocal plots at substrate concentrations of 0.1–2.0 μM. Theophylline (0.2 mM) inhibited cAMP and cGMP phosphodiesterases competitively (figure 1). The K_i-values were 0.36 mM and 0.57 mM respectively.

PG synthetase. The changes are given in percent, because of the variation of the basal activity. The effect of carbenoxolone on the PG synthetase system was biphasic. In the concentration range of 0.1–0.5 mM, a 10–30% stimulation was observed, whereas 1–5 mM caused a concentration-dependent inhibition (figure 2).

Discussion. It has been suggested[6] that carbenoxolone does not act directly on the acid secretion, but stimulates the secretion of the mucus, changes its construction, inhibits pepsin, prolongs the life of migrating epithelial cells and opposes the destruction of the gastric mucosa by bile. There are contradictory findings on the mediator role of cAMP in acid secretion[7], and the possibility that it participates in the secretion of pepsinogen and mucin has not been explored[10]. In man cGMP output increases during maximal pentagastrin stimulation and precedes the increase in acid output[8], supporting the suggestion of Domschke et al.[9] on its role in acid secretion. In dogs cGMP mediates the action of acetylcholine on gastric secretion[15]. Our results that carbenoxolone is a more potent inhibitor of cAMP than of cGMP degrading phosphodiesterase are in agreement with those of Amer[16] and Amer et al.[14] which supports their theory that cAMP is involved in the inhibition and cGMP in the stimulation of gastric secretion. This finding partly explains the anti-ulcer action of carbenoxolone.

PGEs and PGAs inhibit gastric secretion induced by different stimuli[17,18]. PGE_2 has a direct vasodilator effect on the mucosa in gastric perfusion in doses which inhibit secretion but do not affect blood pressure[17]. In addition, PGEs inhibit isosmotic net fluid transport in rabbit gallbladder[19]. This could also contribute to the antisecretory effect of PGE. The concentrations of carbenoxolone needed to stimulate PG synthetase were higher (0.1–0.5 mM) than the level reached in man provided that the drug is evenly distributed in the body. However, there are no data on the concentrations or possible accumulation of this drug in the gastric mucosa.

Kidney medulla was chosen as the source for PG synthetase also because the side-effects of carbenoxolone are associated with electrolyte and water balance. This has been suggested to be a result of aldosterone liberation[20]. On the other hand, the inhibition of PG biosynthesis by carbenoxolone, and thus reduction in kidney microcirculation, could partially explain these side-effects.

In conclusion, we have demonstrated in vitro that carbenoxolone, a synthetic product of glycyrrhizic acid from liquorice root, acts on the degradation of cyclic nucleotides and biosynthesis of PGs which regulate the gastric acid secretion. Although our study does not give the final explanation of the anti-ulcer effect of carbenoxolone, it might explain the therapeutic action of the drug, by influence on cyclic nucleotides, and side-effects, by inhibition of kidney PG-synthetase.

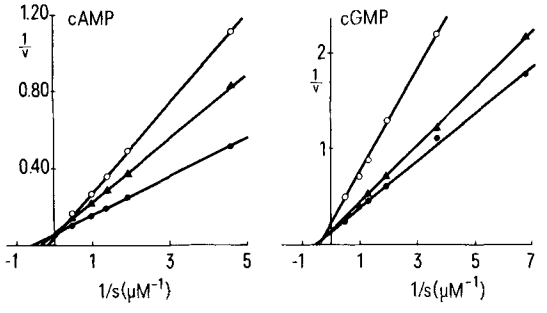

Fig. 1. Double reciprocal plots[21] of the effects of carbenoxolone and theophylline on the rat gastric mucosal phosphodiesterase

V is expressed as $\frac{\text{pmoles substrate hydrolyzed}}{\text{min} \times \text{reaction mixture}}$

(○) the effect of carbenoxolone, (▲) the effect of theophylline, (●) control. The concentrations of carbenoxolone and theophylline were 0.1 mM and 0.2 mM respectively. The effect on cAMP degradation of the left (n = 4). The effect on cGMP degradation on the right (n = 3).

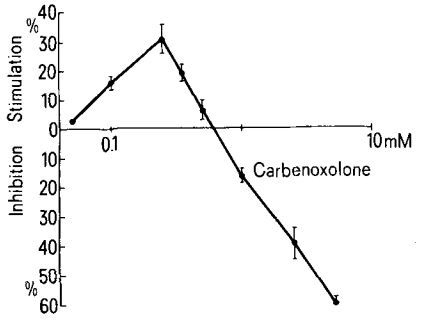

Fig. 2. Dual effect of carbenoxolone on the rabbit kidney medulla prostaglandin synthetase. Means ± SE are given (n = 6–12).

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