Evidence for the existence of another type of histamine H_2-receptor in guinea-pig ileum

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The hypotensive effect of clonidine has been shown partly to be due to stimulation of central histamine H_2-receptors (Karppanen et al 1977). Clonidine may also interact with histamine H_2-receptors in the gastric mucosa (Karppanen & Westermann 1973) and it stimulates the accumulation of cyclic AMP in slices from guinea-pig brain by activation of H_2-receptors (Audiger et al 1976). In the present report inhibition by extremely low concentrations of clonidine of electrically-induced contractions of isolated guinea-pig ileum is described and evidence is presented, that this effect may be due to interference with histamine H_2-receptors probably not identical to the receptors in the heart.

The coaxially stimulated, guinea-pig isolated ileum and the spontaneously beating, guinea-pig isolated atria were used. In the ileum, twitches were induced by supramaximal stimulation with rectangular pulses of 1 ms duration, 20–40 V, at a frequency of 6 Hz. In the atria positive chronotropy was induced by applying histamine in cumulative concentrations (10^{-7}–10^{-4} M).

Clonidine induced a concentration-dependent inhibition of the stimulation-induced twitches in the ileum. A concentration of 10^{-7} M completely abolished the twitches, whereas 10^{-9} M had only a negligible effect. The concentration of clonidine resulting in 50% inhibition was calculated to $6{\cdot}6 \times 10^{-9}$ M. Burimamide (Black et al 1972) concentration-dependently reversed the effect of 3·2–12·5 nM of clonidine (Table 1). 50% reversal of the effect of clonidine was obtained with $2{\cdot}2 \times 10^{-6}$ M of burimamide. Applied in concentrations of 1–10 μM, burimamide itself caused a slight increase in the height of the twitches. Cimetidine, another specific histamine H_2-receptor antagonist (Brimblecombe et al 1975), also reversed the depressive effect of clonidine. However much higher concentrations had to be used in comparison with burimamide. A concentration of $5{\cdot}7 \times 10^{-5}$ M of cimetidine inhibited the effect of clonidine by 50%.

Table 1. Effect of various receptor blockers on clonidine-induced suppression of electrically-stimulated guinea-pig ileum and histamine-induced chronotropy in guinea-pig atria.

Substance	Ileum IC50*	Atria** K_I (mean ± s.e.m.)
Burimamide	$2{\cdot}2 \times 10^{-6}$ M	$7{\cdot}0 \pm 2{\cdot}3 \times 10^{-6}$ M
Cimetidine	$5{\cdot}7 \times 10^{-5}$ M	$1{\cdot}4 \pm 0{\cdot}4 \times 10^{-7}$ M
Diphenhydramine	$>10^{-6}$ M	$>10^{-5}$ M
Phenoxybenzamine	$>4 \times 10^{-7}$ M	
Propanolol	$>1{\cdot}25 \times 10^{-5}$ M	
Methysergide	$>3{\cdot}2 \times 10^{-7}$ M	
Naloxone	$>2{\cdot}5 \times 10^{-8}$ M	

* Reversal of clonidine-induced suppression of the twitches (IC50) dl = $6{\cdot}6 \times 10^{-9}$ M)

** Antagonism of histamine chronotropy. Km for histamine-induced positive chronotropy = $2{\cdot}5 \pm 0{\cdot}2 \times 10^{-6}$ M.

Histamine (10^{-6} M) contracted the guinea-pig isolated ileum. However, after incubation of the ileum with mepyramine (10^{-7}–10^{-6} M) the exitatory effect of histamine was abolished and replaced by an inhibition of the electrically induced twitches. This inhibition was completely reversed by burimamide (5×10^{-6} M) or cimetidine (10^{-4} M). Specific antagonists of histamine H_1-receptors, (diphenhydramine 10^{-8}–10^{-6} M), α- (phenoxybenzamine $1{-}4 \times 10^{-7}$ M) and β-adrenoceptors (propranolol 10^{-7}–$1{\cdot}25 \times 10^{-5}$ M), 5-HT receptors (methysergide 10^{-8}–$3{\cdot}2 \times 10^{-7}$ M) and opiate receptors (naloxone $2{\cdot}5 \times 10^{-8}$ M) had no influence on clonidine-induced suppression of the twitches in concentrations which exhibited distinct antagonistic effect of the respective neurotransmitters (Table 1).

Histamine caused positive chronotropy in the guinea-pig isolated atria, with a K_m of $2{\cdot}5 \times 10^{-6}$ M. Burimamide competitively shifted the histamine concentration-response curves to the right. K_I of burimamide for antagonism of the effect of histamine was calculated to $7{\cdot}0 \times 10^{-6}$ M (Table 1). Cimetidine also antagonized histamine-induced positive chronotropy in a competitive manner, with a K_I of $1{\cdot}4 \times 10^{-7}$ M. Diphenhydramine was not able to antagonize the effect of histamine in concentrations up to 10^{-5} M. Furthermore, clonidine (10^{-8}–10^{-7} M) did not cause positive chronotropy in the atria.

The results obtained with the receptor blockers in the ileum preparation indicate, that clonidine-induced suppression of electrically-induced twitches may be due to stimulation of histamine H_2-receptors. The existence of H_2-receptors in the ileum is furthermore demonstrated by the fact, that after blockade of the H_1-receptors, histamine itself suppresses the electrically-induced twitches, an effect which was completely reversed by specific H_2-receptor antagonists. If the receptors, by means of which clonidine (and histamine) suppress the electrically-induced twitches are "classical" H_2-receptors (Black et al 1972), one would expect the same relative potency between H_2-receptor antagonists in this model and e.g. in isolated atria. However this was not so. In the histamine stimulated atria prepara-

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tion cimetidine was about 50 times as potent as burimamide, which corresponds rather well with previous data, whereas the reverse was true in the ileum (burimamide about 25 times as potent as cimetidine). These differences may possibly be explained by the existence of different histamine H_2-receptors in the atria and in the ileum. This theory is supported by the lack of chronotropic effect of clonidine in the atria.

The generous supply of burimamide and cimetidine by Smith, Kline & French Laboratories is gratefully acknowledged.

July 12, 1978

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Effect of metiamide, a histamine H_2-receptor antagonist on the clonidine-induced decrease in rat brain noradrenaline turnover

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Clonidine is a potent antihypertensive agent which is thought to stimulate central α-adrenoceptors, thereby bringing about a depression of the cardiovascular system (Kobinger & Walland 1967; Schmitt et al 1973). Neurochemically, this action is reflected as a decrease in brain noradrenaline turnover presumably due to α-receptor activation which in turn, by a negative feedback mechanism, decreases the neuronal release of noradrenaline (Andén et al 1970). Recent studies have indicated that metiamide (Karppanen et al 1976; Finch et al 1977) and cimetidine (Finch et al 1977), histamine$_2$-receptor (H_2-receptor) antagonists (Black et al 1972), when administered intracerebroventricularly antagonized clonidine-induced antihypertensive activity in rats. These findings suggest a possible role for H_2-receptor stimulation in the antihypertensive effect of clonidine. Other studies have indicated that clonidine stimulates H_2-receptors in the gastric mucosa (Karppanen & Westermann 1973), heart (Csongrady & Kobinger 1974) and brain (Sastry & Phillis 1976; 1977).

In view of these observations, investigations were carried out to determine whether metiamide antagonizes the effect of clonidine on brain noradrenaline turnover. An ability to cause an alteration of this effect would be indicative of a possible relation between histamine and noradrenaline-containing neurons in the brain.

Male Sprague-Dawley rats (140–160 g, Canadian Breeding Laboratories) were used. The effects on brain noradrenaline turnover were evaluated by studying the decline of brain noradrenaline after treatment with the dopamine β-hydroxylase inhibitor Fla-63, bis-(4-methyl - 1 - homopiperazinyl-thiocarbonyl) disulphide (Regis Chemical Co.) (Andén et al 1972). Animals were injected intracerebrally into the lateral ventricle (Noble et al 1967) with saline or metiamide, and 30 min later intraperitoneally with saline or clonidine. After a further 30 min the rats were injected with saline or Fla-63 and 4 h later they were decapitated, the whole brains quickly removed, rinsed in saline and frozen. Noradrenaline was measured in homogenates of brain prepared in 0·4 M perchloric acid containing 0·1% EDTA and 0·1% ascorbic acid. Noradrenaline was isolated from the homogenate by adsorption onto alumina (Whitby et al 1961), eluted from the alumina with 0·2 M acetic acid and measured flurometrically by the method of Shellenberger & Gordon (1971).

Clonidine hydrochloride and metiamide were gifts from Boehringer Ingelheim Ltd. and Smith, Kline and French Ltd., respectively.

Neither metiamide (0·3 mg kg^{-1}, intraventricularly) nor clonidine (0·1 mg kg^{-1}, i.p.) administered alone altered brain noradrenaline concentrations (Table 1) while treatment with Fla-63 caused a depletion. Clonidine administered before Fla-63 reduced the fall in brain noradrenaline induced by Fla-63; metiamide was ineffective. Pretreatment with metiamide did not alter the clonidine-induced reduction of the fall in brain noradrenaline observed following Fla-63.

The results of this study demonstrate that administration of clonidine, but not metiamide, reduces the deple-

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