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THE ROLE OF β_1- AND β_2-ADRENOCEPTORS IN THE INHIBITION OF GASTRIC ACID SECRETION IN THE DOG

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1 Characterization of the β-adrenoceptors mediating inhibition of gastric acid secretion in the conscious Heidenhain pouch dog has been investigated by determination of the effects of propranolol, (+)-propranolol, practolol and H35/25 on salbutamol and isoprenaline-induced inhibition of gastric acid secretion.

2 The gastric antisecretory effect of salbutamol was significantly blocked by propranolol and H35/25 but not by practolol or (+)-propranolol. The effect of isoprenaline was significantly blocked by propranolol and practolol but not by H35/25 or (+)-propranolol.

3 It is concluded that both β_1- and β_2-adrenoceptors can mediate inhibition of pentagastrin-induced gastric secretion in conscious dogs with a Heidenhain pouch. Salbutamol exerts its antisecretory effect through β_2-adrenoceptors, whereas isoprenaline mediates its effects primarily through β_1-adrenoceptors.

4 The results are discussed with regard to the sub-classification of β-adrenoceptors and to the possible role of adrenoceptors in the physiological control of gastric secretion.

5 In this study it is concluded that the tachycardia induced by isoprenaline or salbutamol is mediated primarily through reflexes activated by β_2-adrenoceptor mediated vasodilatation.

Introduction

Although β-adrenoceptor agonists inhibit pentagastrin-induced acid secretion in the dog (Curwain & Holton, 1972; Daly & Stables, 1977) the β-adrenoceptor sub-type involved has yet to be fully characterized. We have investigated this problem in more detail and the results are presented in this paper.

Receptors can be classified pharmacologically by determination of relative potencies in a series of agonists or by determination of the potencies of competitive antagonists (Furchgott, 1972). Using the latter approach we have sought to characterize the β-adrenoceptors that mediate inhibition of gastric secretion by studying the non-selective β-adrenoceptor agonist isoprenaline (Ekue, Shanks & Zaidi, 1971), the β_2-selective agonist salbutamol (Cullum, Farmer, Jack & Levy, 1969) and their interactions with the β_1- and β_2-adrenoceptor antagonist propranolol (Shanks, 1966), the β_1-selective antagonist practolol (Dunlop & Shanks, 1968) and the β_2-selective antagonist H35/25 (Levy, 1967). (+)-Propranolol, the inactive isomer of propranolol, was used as a control for effects unrelated to β-adrenoceptor blockade (Howe & Shanks, 1966). A preliminary account of some of these results has been given to the British Pharmacological Society (Daly, Long & Stables, 1977).

Methods

Selection of antagonist doses

The doses of the β-adrenoceptor antagonists used in these experiments were those which produced about 15 to 20-fold displacement to the right of the tachycardia or vasodepressor dose-response curve to isoprenaline as appropriate. The intravenous doses of practolol (1 mg/kg) and propranolol (0.1 mg/kg) were obtained from a previous study in our laboratory with the same strain of dog (Daly, Flook & Levy, 1975) by reference to those results where antagonist activity was assessed against isoprenaline-induced tachycardia for practolol and against isoprenaline-induced vasodepression for propranolol. Practolol at this dose markedly antagonized the β_1-adrenoceptor-mediated tachycardia without affecting the β_2-adrenoceptor-mediated vasodepression. Propranolol on the other hand produced clear antagonism of isoprenaline on both systems. As no comparable data were available for H35/25, experiments were carried out in 7 beagles of either sex weighing 6 to 11 kg by the method of Daly *et al.* (1975). The displacement of the isoprenaline vasodepressor and tachycardia dose-response curves was determined following doses of 1, 3 and 10 mg/kg intravenously of H35/25. The dis-

placement of the vasodepressor dose-response curve was used to select a dose for this study.

The selected dose of each antagonist was tested alone in the conscious Heidenhain pouch dog to ensure that it had no significant effect on pentagastrin-induced gastric secretion.

Anti-secretory studies

The method is essentially that of Daly & Stables (1977). Four male beagles (13 to 19 kg) with well established Heidenhain pouches were used. Pentagastrin was infused at doses (1 to 4 μg kg^{-1} h^{-1} i.v.) that produced a 50% maximal secretory response in each dog. The pouch secretion was allowed to drain into a collection vessel which was changed every 15 min. The volume of secretion was measured to the nearest 0.1 ml and an aliquot titrated against 0.1 mol/l NaOH to pH 7 with a Radiometer TTT2 titration system. Acid output was calculated in μmol H^+/min. Heart rate was measured by palpation at 15 min intervals so that the effect of the β-adrenoceptor agonists and antagonists could be monitored on a system with known adrenoceptor characteristics.

In all experiments the β-adrenoceptor agonist and antagonist were administered during a stable plateau of gastric secretion induced by a continuous infusion of pentagastrin.

In an initial series of experiments dose-response curves for inhibition of pentagastrin-induced acid secretion were obtained for isoprenaline and salbutamol. At least three dose levels of each agonist was used in each of the four dogs. The β-adrenoceptor agonists were infused intravenously for 1 h at each dose level. Only one dose level was tested in each experiment. In the main series of experiments the doses of isoprenaline (3 to 10 ng kg^{-1} min^{-1} for 60 min) and salbutamol (100 to 300 ng kg^{-1} min^{-1}) which had been found to produce approximately 70% inhibition of gastric acid secretion were tested at least 3 times in each dog 15 min after intravenous injection of saline, propranolol 0.1 mg/kg, practolol 1.0 mg/kg, H35/25 3.0 mg/kg or (+)-propranolol 0.1 mg/kg. Only one dose of antagonist was tested against the standard dose of either isoprenaline or salbutamol in each experiment. The experiments were carried out in random order.

Results have been calculated as % changes in gastric acid secretion and heart rate by comparing the mean of the two consecutive values at peak drug response with the mean of the four control values preceding the injection of saline or β-adrenoceptor antagonist.

Drugs

The drugs used were (±)-H35/25 (AB Hassle); (−)-isoprenaline bitartrate dihydrate (Ward Blenkinsop & Co); pentagastrin; (±)-practolol; (+)-propranolol; (±)-propranolol (ICI) and (±)-salbutamol (Allen & Hanburys). In the text reference to isoprenaline, salbutamol, H35/25 and practolol means the isomeric form detailed above. Propranolol refers to the (±)-racemate unless specifically designated as (+)-propranolol. Drug doses are expressed as base. All drugs were dissolved in sterile 0.9% w/v NaCl solution (saline) and solutions of isoprenaline contained ascorbic acid 20 μg/ml. The samples of propranolol and practolol were a generous gift from Dr J. Conway, ICI Limited.

Results

Selection of antagonist doses

In the dog anaesthetized with pentobarbitone, H35/25, at a dose of 3 mg/kg, produced a 15.2 ± 5.7 (mean ± s.e.) fold displacement to the right of the isoprenaline vasodepressor dose-response curve and a 3.6 ± 0.7 fold displacement to the right of the isoprenaline-induced chronotropic dose-response curve.

The selected doses of the β-adrenoceptor antagonists were investigated for possible effects on gastric secretion and resting heart rate in the conscious dog (Table 1). Since no marked effects on gastric secretion

Table 1 The effects of propranolol, (+)-propranolol, practolol and H35/25 on gastric secretion and heart rate in conscious dogs with Heidenhain pouches receiving a continuous intravenous infusion of pentagastrin

Parameter	*% change (mean + s.e.) within 75 min of i.v. injection of:*			
	Propranolol 0.1 mg/kg	*(+)-Propranolol 0.1 mg/kg*	*Practolol 1.0 mg/kg*	*H35/25 3.0 mg/kg*
Gastric acid secretion	+19.3 ± 8.4	+10.3 ± 8.5	+2.0 ± 6.1	−1.4 ± 11.6
Heart rate	−9.4 ± 4.8	+1.0 ± 5.0	−9.4 ± 5.4	+18.1 ± 5.9
(n)	(8)	(8)	(8)	(8)

(n) = number of observations.

or heart rate were seen, it was considered valid to use these doses in the main study.

Anti-secretory studies

The anti-secretory responses to isoprenaline and salbutamol started within the first 15 min and reached equilibrium by the end of the 60 min infusion period. The response to isoprenaline recovered considerably within the first 15 min after terminating the infusion of agonist and recovery was complete after 30 to 45 min. The response to salbutamol was more prolonged with recovery starting 15 to 30 min after stopping the agonist infusion and taking 60 to 180 min for full recovery. The results of the interaction studies between the two β-adrenoceptor agonists and the three β-adrenoceptor antagonists on gastric secretion are summarized in Table 2. Propranolol, but not (+)-propranolol, significantly inhibited the anti-secretory actions of isoprenaline and salbutamol showing that the effects of propranolol were due to blockade of β-adrenoceptors and not to membrane stabilizing or local anaesthetic properties. Practolol significantly inhibited the anti-secretory action of isoprenaline but not that of salbutamol, whereas H35/25 significantly inhibited the anti-secretory action of salbutamol, but not that of isoprenaline.

Heart rate studies

In the hour before administration of saline or β-adrenoceptor antagonist the mean resting heart rate from all the interaction experiments was 70.5 ± 0.9 beats/min (mean ± s.e. for 127 experiments). Both isoprenaline (1 to 10 ng $kg^{-1} min^{-1}$) and salbutamol (10 to 300 ng $kg^{-1} min^{-1}$) produced a dose-related tachycardia. The time courses of these increases in heart rate were the same as for inhibition of gastric secretion.

At equivalent anti-secretory dose levels the increase in heart rate produced by isoprenaline was approximately half that produced by salbutamol. The isoprenaline-induced tachycardia was significantly inhibited by propranolol and practolol, but not by (+)-propranolol or H35/25. Salbutamol-induced tachycardia was significantly inhibited by propranolol, practolol and H35/25, but not by (+)-propranolol. These results are shown in Table 3.

Table 2 The effects of isoprenaline and salbutamol on pentagastrin-induced acid secretion after saline, propranolol, (+)-propranolol, practolol or H35/25 in conscious dogs with Heidenhain pouches

Agonist †	*Saline*	*% reduction in acid secretion (mean + s.e.) after i.v.:* *Propranolol 0.1 mg/kg*	*(+)-Propranolol 0.1 mg/kg*	*Practolol 1.0 mg/kg*	*H35/25 3.0 mg/kg*
Isoprenaline	72.2 ± 2.4	47.7 ± 6.5***	67.2 ± 3.1	33.4 ± 3.7***	62.5 ± 4.6
(n)	(14)	(12)	(13)	(12)	(12)
Salbutamol	69.1 ± 4.1	12.6 ± 4.4***	63.5 ± 4.8	61.8 ± 3.9	38.6 ± 4.8***
(n)	(14)	(12)	(12)	(13)	(13)

† Doses as defined in methods.
(n) = number of observations.
Significantly different from control, by *t* test: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3 The effects of isoprenaline and salbutamol on the heart rate of conscious dogs with Heidenhain pouches after saline, propranolol, (+)-propranolol, practolol or H35/25

Agonist †	*Saline*	*% increase in heart rate (mean + s.e.) after i.v.:* *Propranolol 0.1 mg/kg*	*(+)-Propranolol 0.1 mg/kg*	*Practolol 1.0 mg/kg*	*H35/25 3.0 mg/kg*
Isoprenaline	44.5 ± 5.2	1.8 ± 1.4***	31.7 ± 6.2	27.6 ± 6.0*	37.3 ± 7.4
(n)	(14)	(12)	(13)	(12)	(12)
Salbutamol	105.6 ± 7.9	9.0 ± 3.0***	99.7 ± 14.0	73.8 ± 6.1**	33.3 ± 4.1***
(n)	(14)	(12)	(12)	(13)	(13)

† Doses as defined in methods.
(n) = number of observations.
Significantly different from control by *t* test: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Discussion

The purpose of this study was to establish the β-adrenoceptor sub-types that mediate inhibition of pentagastrin-induced acid secretion in the dog and to consider the implication of these findings in regard to the physiological control of gastric secretion.

The inhibition of pentagastrin-induced gastric acid secretion by salbutamol was virtually abolished by propranolol, markedly inhibited by H35/25, but unaffected by practolol. This suggests that salbutamol inhibits gastric secretion by activating β_2-adrenoceptors. This finding was not unexpected in view of the β_2-selective profile exhibited by salbutamol in other systems (Daly, Farmer & Levy, 1971). In contrast, the anti-secretory action of isoprenaline was clearly inhibited by practolol and propranolol, but was unaffected by H35/25. These results suggest that the anti-secretory effect of isoprenaline is mediated exclusively through β_1-adrenoceptors, which is surprising because isoprenaline generally activates both β_1- and β_2-adrenoceptors.

It can be concluded therefore that activation of both β_1- and β_2-adrenoceptors can inhibit gastric secretion. This conclusion is in line with an increasing body of evidence that both β_1- and β_2- adrenoceptors can occur in a single organ or tissue and can mediate the same response, e.g. in trachea (Furchgott, Wakada, Sorace & Stollak, 1975) heart and adipose tissue (Ablad, Borg, Carlsson, Ek, Johnsson, Malmfors & Regardh, 1975). In these circumstances, the number and type of β-adrenoceptor stimulated will depend on the relative affinity and efficacy of the β-adrenoceptor agonist, as well as the relative numbers of the two types of receptor present in the tissue. Thus, the present results are best explained by postulating that the β-adrenoceptor population mediating inhibition of gastric acid secretion is comprised of a large proportion of β_1-adrenoceptors and a small proportion of β_2-adrenoceptors, capable nevertheless, of completely inhibiting pentagastrin-induced acid secretion. The results of this study can then be satisfactorily accommodated within the dual β-adrenoceptor theory of Lands, Arnold, McAuliff, Luduena & Brown (1967).

The effects of the β-adrenoceptor agonists and antagonists on heart rate were essentially as described by other workers, and confirmed that the dogs used in this study responded to these drugs in a conventional manner. Both salbutamol and isoprenaline produced an appreciable tachycardia in these experiments. This was not unexpected since Dunlop & Shanks (1968) have shown that, in conscious dogs, a large proportion of the positive chronotropic response to β-adrenoceptor agonists is due to reflex inhibition of cardiac vagal activity secondary to a fall in blood pressure. The tachycardia to salbutamol was markedly inhibited by the selective β_2-adrenoceptor antagonist H35/25 and the non-selective antagonist propranolol, suggesting that the tachycardia is caused primarily by β_2-adrenoceptor-mediated vasodilatation. There was also a slight but significant inhibition of this tachycardia by practolol which may reflect inhibition of a small reflex increase in sympathetic drive. The tachycardia elicited by isoprenaline was virtually abolished by propranolol but only partially reduced by practolol, which supports the conclusion of Dunlop & Shanks (1968) that isoprenaline, like salbutamol, produces tachycardia largely through β_2-adrenoceptor-mediated vasodilatation. The apparent ineffectiveness of H35/25 may be partly due to a direct stimulant action on heart rate masking its antagonist activity.

The finding that both β_1- and β_2-adrenoceptors can mediate inhibition of gastric secretion may have relevance to the role of the autonomic nervous system in the control of gastric secretion. The parasympathetic nervous system is undoubtedly involved in eliciting and facilitating gastric secretion but there is disagreement about the role and importance of the sympathetic nervous system (Harries, 1956; Sanders, 1976). Although it is well established that noradrenaline can inhibit gastric secretion, this is usually attributed to a reduction in the gastric mucosal blood supply, resulting from activation of α-adrenoceptors (Curwain & Holton, 1972). Noradrenaline can inhibit acetylcholine release from nerves in the intestine by an action on presynaptic α-adrenoceptors (Paton & Vizi, 1969; Kroneberg & Oberdorf, 1974). In the same way noradrenaline may be able to reduce the parasympathetic facilitatory influence on gastric acid secretion. Noradrenaline can also activate β-adrenoceptors and has been reported to have a selective action on β_1-adrenoceptors, whereas adrenaline has a selective action on β_2-adrenoceptors (Lands *et al.*, 1967; Arnold, 1972; Ablad *et al.*, 1975). It is therefore possible to speculate that under normal conditions, gastric acid secretion could be modulated by noradrenaline released from sympathetic nerves acting in three different ways: on postsynaptic α-adrenoceptors to reduce mucosal blood flow, on presynaptic α-adrenoceptors to inhibit acetylcholine release and an additional antisecretory action on β_1-adrenoceptors. Under conditions of stress the circulating blood levels of adrenaline are enhanced by release from the adrenal glands (Callingham, 1967). In these circumstances the actions of the sympathetic nervous system described above would be augmented by an action of adrenaline on β_2-adrenoceptors as well as α-adrenoceptors. Although the role of the sympathetic nervous system and the site of action of β-adrenoceptor agonists as anti-secretory agents have yet to be resolved it is clear from this study that both β_1- and β_2-adrenoceptors can mediate inhibition of gastric secretion.

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