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Stimulation of bicarbonate secretion by α- and β-adrenoceptor agonists in rat caecum in vitro

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Abstract

This study examines the effects of adrenergic drugs on bicarbonate secretion by the rat caecum in vitro. Noradrenaline, phenylephrine but not clonidine, stimulated secretion in a concentration-related manner. Noradrenaline responses were antagonised by alprenolol (20 μM) but not phentolamine (10 μM) whilst phenylephrine was antagonised by phentolamine (10 μM), prazosin (5 μM) but not yohimbine (5 μM), alprenolol or tetrodotoxin (1 μM). Replacement of mucosal Cl^- abolished the phenylephrine response. Combined stimulation with maximum concentrations of phenylephrine and isoprenaline gave a response which was not greater than that to either agonist alone but it did involve both α- and β-adrenoceptors as judged from the effects of alprenolol and phentolamine either alone or combined. Submaximum concentrations of the two agonists did show additive responses. The results show that α_1- but not α_2-adrenoceptor agonists stimulate bicarbonate secretion and may act on the same transport mechanism as β-adrenoceptor agonists. Noradrenaline stimulates via β-adrenoceptors.

Keywords: Bicarbonate secretion; α-Adrenoceptor agonist; α-Adrenoceptor antagonist; β-Adrenoceptor agonist; β-Adrenoceptor antagonist

1. Introduction

When rat caecum in vitro is bathed on the serosal side with a bicarbonate-buffered saline and with an unbuffered saline on the mucosal side, the mucosal solution becomes alkalinised. Previous studies with this preparation have shown that this alkaline flux is due to movement of bicarbonate across the tissue and that about 70% of this is due to an oxygen-dependent transport process; the remainder being diffusion down the concentration gradient for bicarbonate across the tissue (Canfield, 1991; Abdul-Ghaffar, 1993). Part of the oxygen-dependent flux required the presence of chloride on the mucosal side and this component was inhibited by acetylcholine receptor agonists (Canfield and Abdul-Ghaffar, 1991) and stimulated by β-adrenoceptor agonists (Canfield and Abdul-Ghaffar, 1992). As previous reports (Field and McColl, 1973; Racusen and Binder, 1979) have suggested that drugs acting at α-adrenoceptors can influence ion transport in the intestine, we have investigated the effects of α-adrenoceptor agonists both alone and in combination with isoprenaline on bicarbonate secretion in the caecum.

2. Materials and methods

2.1. Tissue preparation

We have used full-thickness tissue preparations. The advantages of this are that the intrinsic nervous system remains intact and that the tissue is subjected to minimal handling thus reducing the risk of the release of local mediators which may influence ion transport mechanisms. Possible disadvantages are that the presence of additional tissue layers may limit the supply of oxygen to the tissue and may interfere with ion movements. Both basal secretion and drug responses are maintained for several hours (Canfield, 1991; Abdul-Ghaffar, 1993) suggesting that there is not progressive tissue hypoxia. We have also performed some experiments with stripped tissue which show that there is no

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difference in time course or magnitude of the response with either bethanechol or isoprenaline compared with full-thickness tissue (Abdul-Ghaffar, 1993).

Normally fed, male Wistar rats (250–350 g) were killed by cervical dislocation. The abdomen was opened by a mid-line incision and the caecum exteriorised, dissected free of mesentery and removed. It was opened and the contents washed away with cold, isotonic saline. Portions of the tissue were tied over the end of a plastic tube (cross-sectional area 1.13 cm^2) with the mucosa facing the tube lumen. Six pieces of tissue were set up in each experiment from 2 animals. The lumen of the tube contained 5 ml of a solution of composition (in mM): NaCl, 136; KCl, 5; $MgSO_4$, 1.2; $CaCl_2$, 2.4 and glucose, 11.7 gassed with 100% O_2. The tube was suspended in a bath containing 30 ml of a similar solution in which 26 mM $NaHCO_3$ replaced an equivalent amount of NaCl. This was gassed with 95% O_2/5% CO_2 (pH 7.4) and maintained at 36°C.

2.2. Measurement of alkalinisation

The unbuffered solution was replaced every 15 min throughout the experiment for determination of HCO_3^- by back-titration with 5 mM HCl using an autotitrator system (ABU 80, Radiometer, Copenhagen). During titration at room temperature the solution was continuously gassed with 95% O_2/5% CO_2. The titration end point was determined as that pH obtained when a sample of unbuffered saline taken directly from the stock reservoir was similarly gassed and this was redetermined several times during an experiment. This method of estimating alkalinisation was validated by adding known amounts of $NaHCO_3$ to 5 ml of unbuffered saline equivalent in amount to that produced by the tissues as described previously (Canfield, 1991).

2.3. Materials

The following were used: alprenolol, bethanechol, tetrodotoxin, phenylephrine, clonidine, prazosin, noradrenaline, isoprenaline, yohimbine and phentolamine. All drugs were obtained from Sigma, Poole, Dorset, UK except phentolamine which was a gift (Ciba Laboratories, Horsham, W. Sussex, UK) and were made up on the day of the experiment and added to the serosal side (noradrenaline stock solutions contained 0.01% ascorbic acid to prevent oxidation). Adrenoceptor antagonists were added to tissues at the peak response to the agonist.

2.4. Expression of results

Tissues were allowed to recover for 60 min before flux measurements began and the unbuffered solution was changed every 15 min during this period. Statistical comparisons were made between the mean steady basal value of the 15 min period immediately preceding the experimental procedure and the final 15 min flux value at the end of the procedure period. Results are expressed as mean ± S.E.M. as μmol cm^{-2} h^{-1} of base. Statistical analysis was performed using either a paired or unpaired Student's t-test as appropriate and P values less than 0.05 were taken as significant. The number of pieces of tissue used in each study is indicated by n.

2.5. Ion substitution

In some experiments Cl^- was excluded from the unbuffered saline in contact with the mucosa being replaced with $NaNO_3$, KNO_3 and $Ca(NO_3)_2$ whilst the other ions were unchanged. When solution changes were made the tissue was washed 3 times with the new mucosal solution before flux measurements recommenced and the titration end point with the new solution was checked and was normally not different from that obtained with Cl^--containing mucosal saline.

3. Results

3.1. Effects of noradrenaline, phenylephrine and clonidine

Fig. 1 shows concentration-response curves for noradrenaline, phenylephrine and clonidine. Each tissue received 3 or 4 concentrations of one agonist on a cumulative basis and the curve for phenylephrine includes all data from the entire study using the maximum concentration (100 μM). Noradrenaline gave a maximum response of 2.7 ± 0.6 μmol cm^{-2} h^{-1} (n = 6) at 0.5 μM; phenylephrine gave a maximum of 1.6 ± 0.1

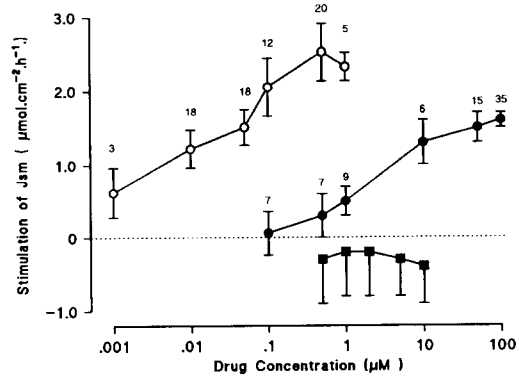

Fig. 1. Concentration-response curves for the stimulation of bicarbonate secretion (Jsm) above basal rate. Open circles = noradrenaline, filled circles = phenylephrine and filled squares = clonidine. Values are means with S.E.M. and the number of observations is shown by each point except for clonidine where n = 6 for all.

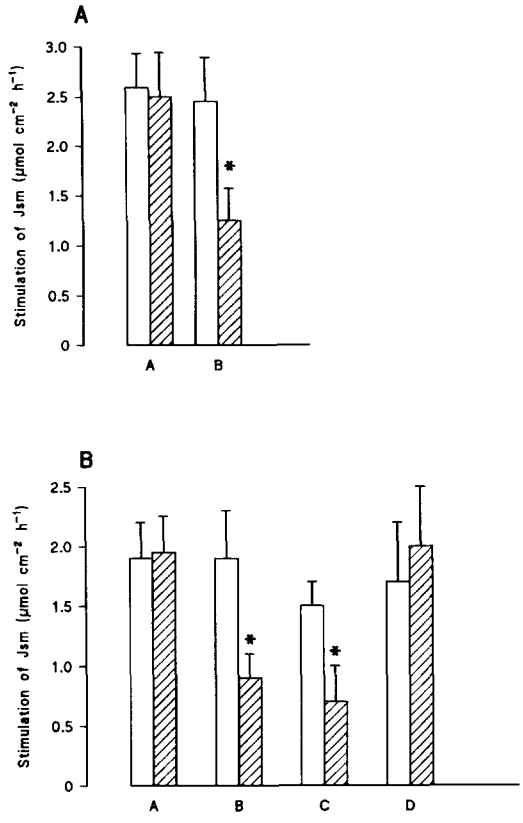

Fig. 2. Histogram showing stimulation of the rate of bicarbonate secretion (Jsm) above basal in response to an agonist (open columns) and the effect of addition of an antagonist at the peak of response (shaded columns). Values are means + S.E.M. and * indicates a P value < 0.05 for the difference between means. 2A: Agonist was noradrenaline (0.5 μM) and antagonists were A = phentolamine (10 μM) and B = alprenolol (20 μM). $n = 10$ for both. 2B: Agonist was phenylephrine (50 μM) and antagonists were A = alprenolol (20 μM), B = prazosin (5 μM), C = phentolamine (10 μM) and D = yohimbine (5 μM). $n = 6$ for all.

μmol cm^{-2} h^{-1} ($n = 35$) at 100 μM whilst clonidine had no significant effect on secretion up to 10 μM. To avoid any possibility that cumulative concentrations of clonidine had desensitised the tissue, a further 9 tissues were exposed to a single concentration of 10 μM resulting in an increase of 0.2 ± 0.6 μmol cm^{-2} h^{-1}.

3.2. Effects of adrenoceptor antagonists

Fig. 2 shows the effects of various antagonists on these responses. The response to noradrenaline was significantly reduced by alprenolol but not by phentolamine suggesting that it was mediated by an action at β- rather than α-adrenoceptors. The response to phenylephrine was significantly reduced by phentolamine and prazosin but not by yohimbine or alprenolol. The antagonists alone had no significant effect on basal secretion (Canfield and Abdul-Ghaffar, 1992; Abdul-Ghaffar, 1993).

3.3. Effects of ion substitution and tetrodotoxin

Removal of Cl^- from the mucosal saline abolished the response to phenylephrine (Fig. 3) but pre-incubation of tissues with tetrodotoxin (1 μM) for 45 min had no effect: control response 1.4 ± 0.3 ($n = 5$); with tetrodotoxin 1.4 ± 0.3 ($n = 6$) μmol cm^{-2} h^{-1}.

3.4. Combined stimulation with phenylephrine and isoprenaline

As both α- and β-adrenoceptor agonists appear to stimulate a transport mechanism which is dependent on mucosal Cl^-, the effects of both types of agonist together were examined. In Fig. 4A, tissues were exposed to a sub-maximal concentration of phenylephrine (10 μM) and when the response had reached a plateau (1.47 ± 0.3 μmol cm^{-2} h^{-1}), a submaximal concentration of isoprenaline (0.1 μM) was added which increased the response to 2.41 ± 0.44 μmol cm^{-2} h^{-1}. A subsequent further addition of isoprenaline to give a maximum concentration (1 μM) resulted in a small, non-significant increase to 2.5 ± 0.5 μmol cm^{-2} h^{-1} ($n = 6$). Applying the agonists in the reverse order produced similar results (data not shown). When the experiments were repeated using maximum concentrations of the agonists (isoprenaline 1 μM; phenylephrine 50 μM), phenylephrine did not increase the response (Fig. 4B). This lack of summation of responses with maximum concentrations was confirmed in a further experiment in which tissues were randomly allocated to one of three treatments: isoprenaline alone (0.5 μM), phenylephrine alone (100 μM) or isoprenaline plus phenylephrine simultaneously. The resulting responses were: isoprenaline = 1.73 ± 0.3, phenylephrine = 1.45 ± 0.3 and isoprenaline + phenylephrine = 1.4 ± 0.2 μmol cm^{-2} h^{-1} ($n = 8$ for each group).

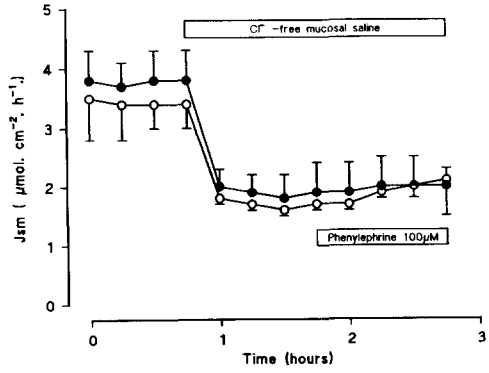

Fig. 3. Effect of replacement of mucosal Cl^- by NO_3^- on the rate of bicarbonate secretion (Jsm) and the response to phenylephrine (open symbols, $n = 6$) compared with control (filled symbols, $n = 5$). Values are means with S.E.M. There were no significant differences between the two groups.

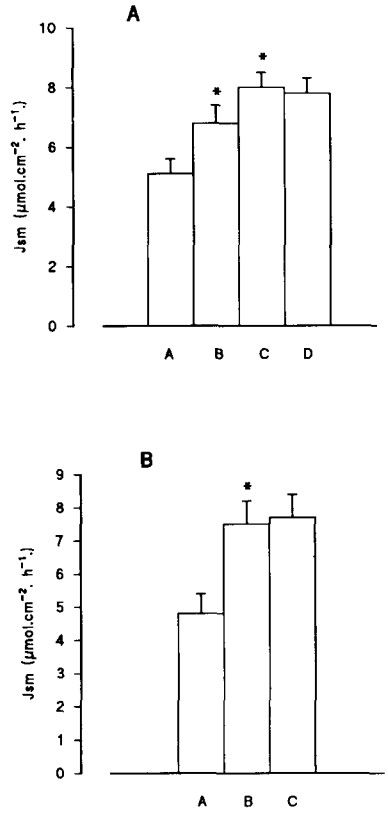

Fig. 4. Histogram of the effects of stimulation of bicarbonate secretion (Jsm) by sequential addition of phenylephrine and isoprenaline together. Values are means + S.E.M. and * indicates $P < 0.05$ compared with preceeding value. 4A: Sub-maximal drug concentrations. A = initial basal rate, B = addition of phenylephrine (10 μM), C = addition of isoprenaline (0.1 μM) in the continued presence of phenylephrine, D = increasing isoprenaline to maximum (1 μM). $n = 6$. 4B: Maximal drug concentrations. A = initial basal rate, B = addition of isoprenaline (1 μM), C = addition of phenylephrine (50 μM) in the continued presence of isoprenaline. $n = 5$.

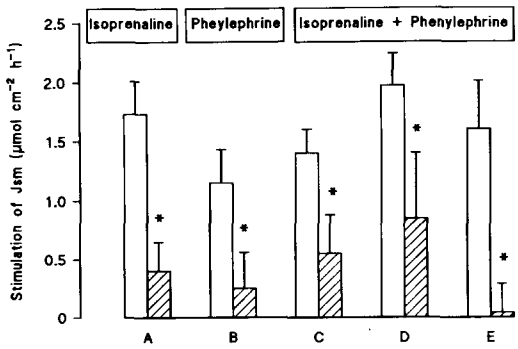

Fig. 5. Histogram showing the effects of antagonists on stimulation of bicarbonate secretion (Jsm) above basal by either isoprenaline (0.5 μM), phenylephrine (100 μM) or isoprenaline + phenylephrine together. Values are means + S.E.M., $n = 6$–8. Open columns show the effect of agonist and shaded columns of addition of the antagonist at the peak of the response and * indicates $P < 0.05$ compared with agonist alone. Antagonists were A and C = alprenolol (20 μM), B and D = phentolamine (10 μM) and E = alprenolol + phentolamine.

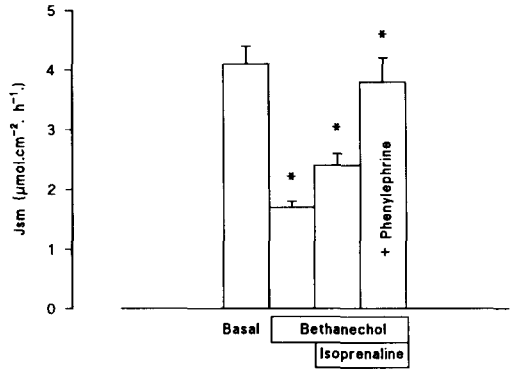

Fig. 6. Histogram showing the effect of bethanechol (250 μM) on basal rate of bicarbonate secretion (Jsm) and responses to maximum concentrations of isoprenaline (1 μM) and phenylephrine (100 μM). Values are means + S.E.M. and $n = 6$ and * indicates $P < 0.05$ compared with preceding value.

3.5. Effects of antagonists on combined stimulation

To substantiate that both α- and β-adrenoceptors were activated during combined stimulation with isoprenaline + phenylephrine, the effects of adrenoceptor antagonists were also examined (Fig. 5). Both alprenolol and phentolamine individually reduced the response to the combined agonists whilst application of both antagonists together abolished the combined agonist response. The results suggest that the individual antagonists produced a smaller percent change in response in the combined stimulation tissues than when they were acting only against stimulation by their 'own' agonist but these differences did not achieve statistical significance.

In no experiment with joint stimulation with isoprenaline and phenylephrine did the response significantly exceed that obtained with a maximum concentration of either agonist alone. This may indicate that the transport mechanism had a limiting maximum rate. In an attempt to test this, tissues were first treated with bethanechol (250 μM) which inhibits that part of basal secretion which depends on mucosal Cl^- (Canfield and Abdul-Ghaffar, 1991). In the continued presence of bethanechol, isoprenaline was added (1 μM) giving a response of 0.6 ± 0.1 μmol cm^{-2} h^{-1} (Fig. 6). Subsequent addition of phenylephrine (100 μM) increased the response by a further 1.4 ± 0.2 giving a combined response of 2.2 ± 0.3 μmol cm^{-2} h^{-1} ($n = 6$). At this point, the total HCO_3^- flux (3.75 ± 0.4 μmol cm^{-2} h^{-1}) was similar to the pre-bethanechol basal rate (4.1 ± 0.25 μmol cm^{-2} h^{-1}).

4. Discussion

Noradrenaline modifies ion transport in rat large intestine by acting at both α- and β-adrenoceptors

(Racusen and Binder, 1979). It was included in the present study in the hope of comparing its dual effect on both types of receptor with the actions of receptor-specific agonists to determine if summation of α and β responses occurred. Even in the absence of uptake inhibitors, it was a potent stimulant of bicarbonate secretion, comparable with earlier work with isoprenaline (Canfield and Abdul-Ghaffar, 1992). However, the antagonist studies indicated that it was acting only at β-adrenoceptors. The apparent lack of effect of noradrenaline on the α-adrenoceptors remains puzzling. These may require higher concentrations of noradrenaline for activation but, given the fact that the β-adrenoceptor-mediated effects where already reduced at 1 μM (Fig. 1) and the lack of any selective antagonist for β_3-adrenoceptors, this was not pursued further.

Phenylephrine but not clonidine also stimulated bicarbonate transport pointing towards an effect via α_1- rather than α_2-adrenoceptors and the results with prazosin and yohimbine supported this. No attempt was made to estimate pK_B values for the antagonists. This type of analysis requires the assumption that the drug concentration at the receptor is the same as that in the bathing fluid. Black and co-workers (Angus et al., 1980; Angus and Black, 1979) have shown that this condition is not achieved in secretory epithelia and consequently results in anomalous pK_B estimates.

It has been widely reported in a number of species that adrenergic agonists stimulate an increase in NaCl uptake and a reduction in short circuit current in both small and large intestine in vitro via an action on α_2-adrenoceptors located either on the enterocyte or submucosal nervous system (Field and McColl, 1973; Racusen and Binder, 1979; Sundaram, 1993; Dharmsathaphorn et al., 1984; Chang et al., 1982, 1983a,b; Hildebrand et al., 1993; Hildebrand and Brown, 1992; Sellin and De Soignie, 1987; Durbin et al., 1982). None of these publications indicates any effects mediated by α_1-adrenoceptors. An exception to this has been reported in porcine distal colon (Traynor et al., 1991) where noradrenaline stimulated a decrease in Na absorption and an increase in both short circuit current and Cl secretion. These effects were inhibited by prazosin but not yohimbine.

There is little published information on the actions of adrenoceptor agonists on intestinal bicarbonate secretion. Adrenaline either abolished this or resulted in bicarbonate absorption in rabbit ileum and proximal colon (Dietz and Field, 1973; Smith et al., 1985; Sullivan and Smith, 1986). The receptor type was not characterised and it is unclear from these studies whether these effects were the result of a direct action on a bicarbonate transport mechanism or were a consequence of stimulation of apical Na^+/H^+ exchange which acidified the lumen and neutralised the bicarbonate. Noradrenaline had no effect on basal bicarbonate secretion in porcine distal jejunum whilst in porcine distal colon (Traynor et al., 1991) it was suggested that the effects of noradrenaline were consistent with a stimulation of luminal $NaHCO_3$ co-transport. However, bicarbonate fluxes were not directly measured in that study. There was no effect of α-adrenoceptor agonists on in vitro bicarbonate secretion by amphibian (Garner et al., 1984) or rat (White, 1987) duodenum and noradrenaline inhibited bicarbonate secretion in rabbit antrum (Fromm et al., 1976). The present study appears to be the first to show an in vitro effect of α_1-adrenoceptors on intestinal bicarbonate secretion.

The lack of effect of tetrodotoxin suggests that phenylephrine may act directly on enterocytes and, like isoprenaline (Canfield and Abdul-Ghaffar, 1992), its effect required the presence of mucosal Cl^- for stimulation of bicarbonate secretion suggesting that both α- and β-adrenoceptors may influence an apical HCO_3^-/Cl^- exchange mechanism, possibly the same mechanism. Combined stimulation with isoprenaline and phenylephrine with submaximal, but not maximal, concentrations showed summation of effects but we were never able to demonstrate a response to combined α and β stimulation which was significantly greater than the response to a maximal concentration of either agonist alone. This might indicate that there was a rate limiting step in the secretory process. If this were of metabolic origin then reducing initial basal demand might permit demonstration of an enhanced response at maximal concentrations. This was attempted pharmacologically by using bethanechol. Although the responses in the presence of bethanechol were additive, the final rate of secretion was not enhanced above that previously obtained with either agonist alone. The response to isoprenaline was much reduced in the presence of bethanechol and this may indicate that the approach was invalid and that these two drugs do not simply have antagonistic actions on the transport process. An alternative explanation is that some basolateral or apical step in the transport process becomes maximally activated and rate limiting. The studies with antagonists (Fig. 5) do not support this as an antagonist-induced reduction in the contribution of either α- or β-adrenoceptor activation to the combined response was associated with a reduction of secretion. Clearly, the non-antagonised receptor type could not fully activate the transport mechanism under these conditions and this may indicate some mutual interaction between the two receptor type pathways. The only other intestinal transport data on joint stimulation of α- and β-adrenoceptors relate to NaCl transport in the rat distal colon (Racusen and Binder, 1979) where it was reported that the action of noradrenaline (which involved both α- and β-adrenoceptors) was the same as that

seen with isoprenaline suggesting that stimulation of both receptor types did not enhance the response.

In summary, activation of α-adrenoceptors stimulated bicarbonate secretion in the rat caecum and this is only the second report of an action of this type of receptor on intestinal ion transport. The effect of simultaneous stimulation of both α- and β-adrenoceptors warrants further investigation.

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