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Characterization of receptors on postganglionic cholinergic neurons in the guinea-pig isolated ileum

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Dopamine, apomorphine, noradrenaline and isoprenaline reduced the response of the isolated guinea-pig ileum to exogenous acetylcholine by a maximum of 40%. Propranolol reversed this inhibition whilst phentolamine and pimozide were ineffective, suggesting that the drugs were acting on a post-synaptic β-adrenoceptor. The same agonists were more effective as inhibitors of the response to transmural electrical stimulation of the ileum, lower doses producing almost complete inhibition. This inhibition was partially antagonized by phentolamine, pimozide and propranolol. Clonidine proved to be the most potent inhibitor of the response to transmural electrical stimulation, whilst phenylephrine was ineffective. pA_2 determinations showed that phentolamine was a potent antagonist of clonidine but a weak antagonist of apomorphine whilst for pimozide the opposite was true. The results suggest that there are two populations of prejunctional receptors on the cholinergic nerves innervating the smooth muscle of the guinea-pig ileum. One receptor is similar to a classical prejunctional α-adrenoceptor and the other resembles a central dopamine receptor.

Apomorphine is an agonist at central dopamine receptors (Ernst 1967) and it also induces vomiting in animals other than rodents by an action on the chemoreceptor trigger zone, (Borison & Wang 1953). Conversely, typical dopamine receptor antagonists, e.g. chlorpromazine and haloperidol are effective in protecting against apomorphine-induced emesis (Carlsson & Lindqvist 1963). However, metoclopramide, an antagonist of dopamine, which is used clinically as an antiemetic (Peringer et al 1975) has few central effects, and domperidone, which lacks effect on the nigrostriatal dopaminergic system is also an antiemetic (Reyntjens et al 1978). Therefore the possibility arises that there is a peripheral component in the action of these drugs.

Large amounts of dopamine are present in the gastrointestinal mucosa especially in the stomach (Hokanson 1970) and it has been suggested by Thorner (1975) that dopamine may modulate cholinergic transmission in the gastrointestinal tract. The recently developed concept of prejunctional receptors which can modulate neurotransmitter release (Endo et al 1977) could provide a possible target site for these drugs in the periphery. Kosterlitz & Watt (1965) suggested that there may be prejunctional α-adrenoceptors on the cholinergic neurons innervating the smooth muscle of the ileum; it is possible that dopamine could act on these receptors. Alternatively it is possible that another population of receptors exist which could be specifically sensitive to dopamine.

Therefore, the effects of dopamine and other agonists and antagonists were examined on the guinea-pig isolated ileum preparation stimulated either by added acetylcholine or by transmural electrical stimulation, to seek for any evidence of modulation of transmitter release and also to identify the receptors involved. Some preliminary results have been communicated to the British Pharmaceutical Conference (Ennis et al 1977).

METHODS

Guinea-pigs of either sex, 300–500 g, were killed by a blow to the head. The ileum was removed except for the final 10 cm. A strip of mid-ileum, 2–3 cm in length was suspended in a 20 ml organ bath in Krebs solution, maintained at 37 °C and aerated with 5% CO_2 in oxygen. The contractions were measured isometrically by means of a 2 oz Ether transducer connected to a two channel Rikadenki potentiometric recorder.

The preparations were stimulated either by exogenous acetylcholine or by passing single electrical pulses (0·5 ms) supramaximal voltage (80 V) at a frequency of 0·2 Hz through stainless steel electrodes. All drugs were added directly to the organ bath after the preparations had been allowed to equilibrate for at least 30 min. Solutions of dopamine and noradrenaline contained ascorbic acid to prevent oxidation.

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With the transmural electrically stimulated ileum, pA_2 determinations were made for phentolamine and pimozide against a range of agonists using the rapid pA_2 method described by Schild (1947). These experiments were performed in the presence of propranolol (10^{-6} M).

Drugs and drug solutions

Acetylcholine chloride (BDH), apomorphine (Janssen pharmaceutica), clonidine sulphate (Boehringer Ingelheim), cocaine hydrochloride(MacFarlan-Smith), dopamine hydrochloride (Koch–Light), isoprenaline sulphate (BDH), noradrenaline bitartrate (Koch–Light), phentolamine mesylate (BDH), pimozide (Janssen pharmaceutica), propranolol hydrochloride (Inderal) (ICI), phenylephrine hydrochloride (Boehringer Ingelheim). Concentrations are expressed as molar in the bath medium.

Statistical analysis

All results are expressed as means ± standard errors. Significance of the differences between groups was calculated using a two-tailed Mann–Whitney U test. Unless otherwise stated the significance level was $P < 0.05$.

RESULTS

Exogenous acetylcholine

Log concentration-effect curves were obtained for acetylcholine on the isolated guinea-pig ileum preparation. From these curves a concentration which produced 50% of the maximum possible response was chosen (EC50) and used in drug antagonism studies. The response obtained to this dose was designated as 100%. All subsequent responses were expressed as a percentage of this response.

The amplitude of contraction of the ileum preparation elicited by the EC50 concentration (5×10^{-8} M acetylcholine) was reduced by pre-incubation of the tissue for 2 min with increasing concentrations of dopamine, apomorphine, noradrenaline or isoprenaline in a dose related manner. The inhibition never exceeded 40% of the maximum inhibition possible, even when extremely high concentrations (i.e. up to 10^{-4} M) of the compounds were used (Fig. 1).

The effect of a range of antagonists on the inhibitory effects of dopamine, apomorphine, noradrenaline and isoprenaline was tested. Phentolamine was ineffective up to 10^{-5} M, pimozide was ineffective up to 2×10^{-8} M but propranolol showed a small effect at 5×10^{-7} M and at 3×10^{-6} M the inhibition was completely abolished (Fig. 2). At these doses there was no effect on the control response to acetylcholine.

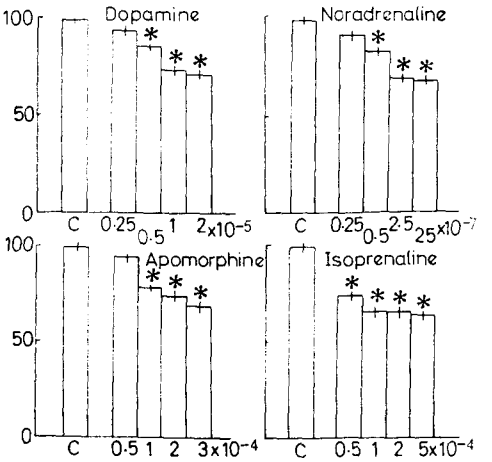

FIG. 1. Effect of dopamine, apomorphine, noradrenaline and isoprenaline on the response (ordinate: % response) of the isolated guinea-pig ileum to exogenous acetylcholine. The control response to an EC50 of acetylcholine is designated 100% (C). Each column represents the mean for 8 determinations. Vertical bars indicate standard error. Abscissa: Inhibitor concentration (M).
* Significant inhibition of control response $P < 0.05$.

Transmural electrical stimulation

Responses to transmural electrical stimulation were elicited by repetitive stimulation at 0·2 Hz using a supramaximal voltage. This response was designated as 100%. The contractions were abolished by atropine (10^{-9} M) and tetrodotoxin ($3{\cdot}1 \times 10^{-7}$ M). Incubation of ileal tissue with either noradrenaline, dopamine, apomorphine or isoprenaline produced a dose-dependent inhibition of the contraction elicited by transmural electrical stimulation. Each drug was left in contact with the tissue until equilibrium blockade had been reached. It is this equilibrium level for each concentration that is shown in Fig. 3, expressed as a percentage of the pre-incubation responses. Dopamine was effective over the range of 5×10^{-6} to 10^{-4} M, apomorphine from 5×10^{-6} to 5×10^{-5} M, noradrenaline from 5×10^{-8} to 1×10^{-5} M and isoprenaline from 5×10^{-7} to $2{\cdot}5 \times 10^{-4}$ M. All the drugs were capable of producing almost complete inhibition of the response to transmural electrical stimulation.

The effect of cocaine (6×10^{-6} M) on the inhibition of transmural electrical stimulation produced by noradrenaline and dopamine was also tested. The mean pD_2 values ± standard errors for noradrenaline and dopamine in the the presence of

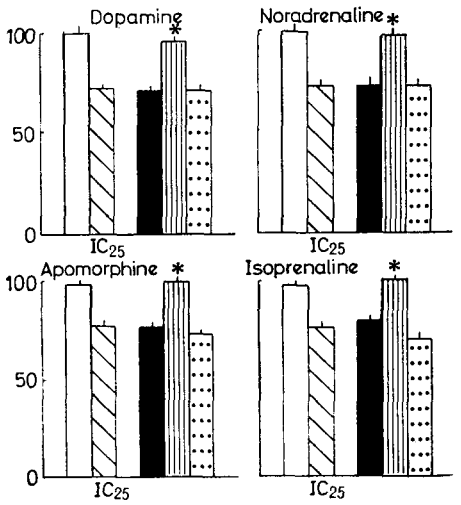

FIG. 2. Effect of antagonist drugs on dopamine-, apomorphine-, noradrenaline- and isoprenaline-induced inhibition of the response (ordinate: % response) of the isolated guinea-pig ileum to exogenous acetylcholine. Open column is the response to EC50 acetylcholine designated 100%. Hatched column is the response in the presence of agonist in a concentration causing 25% inhibition of acetylcholine (IC25). Closed column inhibitor plus phentolamine (1×10^{-5} M). Striped column inhibitor plus propranolol (3×10^{-6} M). Dotted column inhibitor plus pimozide (2×10^{-8} M). Each point is the mean of at least 4 determinations. Vertical bars indicate s.e. mean. * Significant antagonism of IC25 $P < 0.05$.

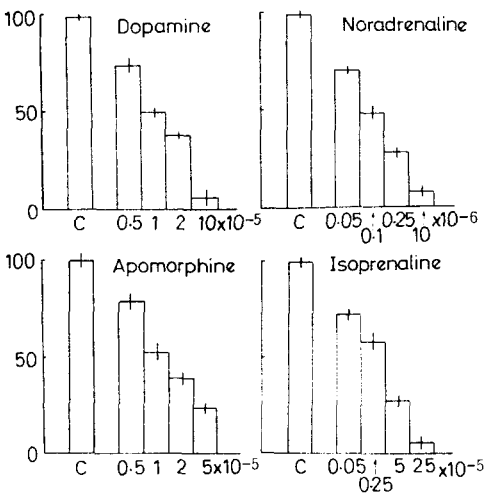

FIG. 3. Effect of dopamine, apomorphine, noradrenaline and isoprenaline on the response (ordinate: % response) of the isolated guinea-pig ileum to transmural electrical stimulation. The control response to transmural electrical stimulation is designated 100% (C). Each column represents the mean of 8 determinations. Vertical bars indicate standard error. Abscissa: Inhibitor concentration (M). Significant antagonism of the control response occurred at each concentration $P < 0.05$.

cocaine were 7.10 ± 0.27 and 5.10 ± 0.16 respectively. These were not significantly different from the values obtained in the absence of cocaine (Table 1).

The effect of antagonists on the inhibition of the response to transmural electrical stimulation was tested in a manner analogous to that for exogenous acetylcholine. Propranolol (3×10^{-6} M) was an effective antagonist but was incapable of completely reversing the inhibitory effects of dopamine, apomorphine, noradrenaline and isoprenaline. From such dose ranging studies, sample doses of phentolamine and pimozide were chosen which were equieffective against noradrenaline, these were phentolamine (10^{-6} M) and pimozide (10^{-8} M). This dose of pimozide appeared to be more effective than the dose of phentolamine in antagonizing dopamine and apomorphine (Fig, 4) but the difference was only statistically significant for apomorphine. These doses of antagonists on their own had no effect on the control response to transmural electrical stimulation.

Further analysis of the inhibition of the response to transmural electrical stimulation was made by increasing the range of drugs used to include clonidine and phenylephrine. Table 1 shows the relative orders of potency of the agonists tested and it can be seen that clonidine was by far the most potent compound whilst phenylephine hardly produced any inhibition even at doses greater than 10^{-4} M.

A more detailed examination of two of the antagonists was made using the pA_2 determination devised by Schild (1947). The antagonists chosen were phentolamine and pimozide and the determinations were performed in the presence of propranolol (10^{-6} M) to exclude interference at the presumed postjunctional β-adrenoceptor. Because of the lack of effect of phenylephrine it was not possible to obtain pA_2 values against this agonist.

Table 2 shows the pA_2 values for phentolamine and pimozide against dopamine, noradrenaline,

Table 1. Negative log EC50 molar concentrations of modifying drugs (pD_2) as inhibitors of the response of the guinea-pig isolated ileum to transmural electrical stimulation. Each figure represents the mean of at least 4 determinations.

Agonist	Mean $pD_2 \pm$ s.e.
Clonidine	8.0 ± 0.29
Noradrenaline	7.0 ± 0.52
Dopamine	5.0 ± 0.39
Apomorphine	4.9 ± 0.45
Phenylephrine	<4.0

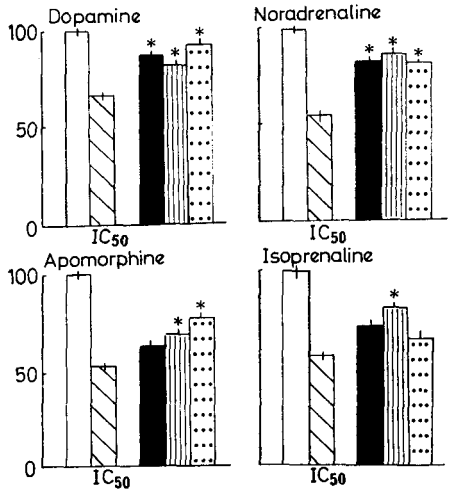

FIG. 4. Effect of antagonist drugs on dopamine-, apomorphine-, noradrenaline- and isoprenaline-induced inhibition of the response (ordinate: % response) of the isolated guinea-pig ileum to transmural electrical stimulation. Open column is the response to transmural electrical stimulation designated 100%. Hatched column is the response in the presence of inhibitor in a concentration causing approximately 50% inhibition (IC50). Closed column inhibitor plus phentolamine (1×10^{-6} M). Striped column inhibitor plus propranolol (3×10^{-6} M). Dotted column inhibitor plus pimozide (1×10^{-8} M). Each point is the mean of at least 4 determinations, vertical bars indicate s.e. mean. * Significant antagonism of the IC50 $P < 0.05$.

apomorphine and clonidine and it can be seen that phentolamine was a potent antagonist of clonidine but a weak antagonist of apomorphine. Pimozide was equally effective against noradrenaline, dopamine and apomorphine yet inactive against clonidine. A value for the pA_2 for pimozide against clonidine was not obtained because at concentrations above 10^{-7} M pimozide was seen to produce a non-specific antagonism of the responses to transmural electrical stimulation.

DISCUSSION

From the experiments using exogenous acetylcholine as the stimulus for contraction, in which the catecholamines and related drugs only produced 40% inhibition and the only effective antagonist was propranolol, it could be considered that the agonists were acting on a postsynaptic β-adrenoceptor. However, since noradrenaline was more potent than isoprenaline, this receptor may not be a classical β-adrenoceptor or may more closely resemble a β_1-adrenoceptor (Lands et al 1967; Moore & O'Donnell 1970).

Table 2. pA_2 values for phentolamine and pimozide in the presence of propranolol (3×10^{-6} M) as antagonists of the inhibition of the response of isolated guinea-pig ileum to transmural electrical stimulation induced by modifying drugs.

Agonist	Mean $pA_2 \pm$ s.e. Phentolamine	Pimozide
Clonidine	*9·28 ± 0·05	* /
Noradrenaline	8·34 ± 0·18	8·29 ± 0·10
Dopamine	8·79 ± 0·09	8·49 ± 0·11
Apomorphine	*7·64 ± 0·05	*7·96 ± 0·05

* Significantly different from noradrenaline pA_2 value. $P < 0.05$. Mann–Whitney U test two tailed.
/ No antagonism within the range known to be specific ($pA_2 < 7.00$).

When the ileum was stimulated by transmural electrical stimulation, phentolamine, pimozide and propranolol all had some effect on the inhibition produced by dopamine, noradrenaline, apomorphine and isoprenaline. Thus inhibition of the response to transmural electrical stimulation involved a different population of receptors from those modifying the response to exogenous acetylcholine. The most likely explanation was that these drugs act on different receptors to modulate acetylcholine release because the tissues were more sensitive to the effects of modifying drugs when they were tested against transmural electrical stimulation and the antagonists phentolamine and pimozide were only effective against the inhibition of transmural electrical stimulation.

In a comparison of either relative agonist potencies or when pA_2 values are determined it is essential that the agonist is not being removed from the biophase by inactivation processes such as uptake$_1$ (Furchgott 1972). Therefore the pD_2 values for noradrenaline and dopamine in the presence of cocaine were compared with their respective pD_2 values in the absence of cocaine. Cocaine, in a concentration known to be effective against uptake, produced no significant change in pD_2. Therefore, uptake in this tissue appears not to be a significant site of loss for the catecholamines and therefore the experimental design in the absence of cocaine is valid. This gives an advantage because the possibility that uptake inhibitors also act on presynaptic receptors can not be excluded and, if they are required, an extra complication in interpretation is introduced.

Experiments with clonidine and phenylephrine produced an order of potency with clonidine as the most potent and phenylephrine as the least potent

compound tested, confirming reports by Drew (1977) and Wikberg (1977). This relative order of potency suggests that a prejunctional α-adrenoceptor is involved (Starke, 1972; Starke et al 1974; Endo et al 1977).

Only concentrations of antagonists shown to have no effect against the response to acetylcholine and transmural electrical stimulation have been quoted in the pA_2 determinations. However, when pimozide was tested againt clonidine the concentration required approached the non-specific range before demonstrable antagonism occurred.

The pA_2 values obtained in this study are either similar to, or higher than previously reported values indicating a greater specificity of the antagonist for the receptor (van Rossum 1965; Green & Fleming 1968; Gulati et al 1968; Kohli 1969; Kelly 1971).

The pA_2 determinations for phentolamine like the relative agonist potencies, indicate the presence of prejunctional α-adrenoceptors since phentolamine was most potent against clonidine and least effective against apomorphine. This is what would be expected if clonidine was acting on an α-adrenoceptor and apomorphine was not. However, pimozide was more effective against apomorphine than clonidine, again suggesting that clonidine and apomorphine were not acting on the same type of receptor. The simplest explanation of the results is that clonidine was stimulating a classical prejunctional α-adrenoceptor and that the other receptor, being sensitive to apomorphine and pimozide, resembled a central dopamine receptor. The middle of the range pA_2 values for noradrenaline and dopamine may suggest that these agonists can stimulate both type of receptor.

Thus it would appear that there are postsynaptic receptors with some similarity to β-adrenoceptors on the smooth muscle cells of the guinea-pig ileum and a prejunctional α-adrenoceptor on the cholinergic neurons innervating the smooth muscle cells. This supports the findings of others (Kosterlitz et al 1970; Paton & Vizi 1969; Wikberg 1977). However, our results also suggest there is a second prejunctional receptor which can modulate cholinergic transmission in the ileum. This appears to resemble a central dopamine receptor but requires further study to more precisely characterize it.

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