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MECHANISM OF ACTION OF DOPAMINE ON THE GUINEA-PIG GASTRO-OESOPHAGEAL JUNCTION *in vitro*

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1 The effect of dopamine on longitudinal muscle strips of the guinea-pig isolated gastro-oesophageal junction was compared with the response obtained to phenylephrine, isoprenaline and clonidine. Phenylephrine (5×10^{-7} to 5×10^{-5} M) produced a dose-related contraction, whilst dopamine (10^{-6} to 10^{-4} M) and isoprenaline (5×10^{-7} to 2×10^{-5} M) produced dose-related relaxations. Clonidine was ineffective in doses up to 10^{-5} M. 5-Hydroxytryptamine (5-HT) produced a contraction.

2 Phenylephrine was antagonized by α_1-adrenoceptor antagonists but unaffected by β-adrenoceptor antagonists, whilst the opposite was the case for isoprenaline. A mixture of α- and β-adrenoceptor antagonists was required to inhibit completely dopamine-induced relaxations. 5-HT (3×10^{-7} M) was specifically antagonized by methysergide (3×10^{-6} M).

3 pA_2 values for a range of α-adrenoceptor and dopamine receptor antagonists were determined against dopamine and phenylephrine. The relative order of potency of the antagonists was the same for both antagonists and was prazosin > spiroperidol > phentolamine > domperidone > haloperidol, with pimozide and metoclopramide being inactive.

4 Tyramine caused dose-related relaxations of the gastro-oesophageal strips which were susceptible to the same range of antagonists as dopamine.

5 Cocaine (6×10^{-6} M) and desmethylimipramine (3×10^{-7} and 10^{-6} M) reduced the relaxations induced by dopamine and tyramine but there were quantitative differences in the antagonism.

6 Tissue from reserpine pretreated guinea-pigs was insensitive to tyramine but the response to dopamine was only partly reduced.

7 Histological examination of the strips revealed the presence of smooth muscle but only a sparse adrenergic innervation.

8 The results suggest that dopamine acts partly indirectly and partly directly on postjunctional α- and β-adrenoceptors. There is no evidence for an action on specific dopamine receptors.

Introduction

Neuroleptic compounds, such as phenothiazines and butyrophenones have long been used in the treatment of nausea and vomiting. However, these compounds have sedative and extrapyramidal side effects and have been assumed to exert their anti-emetic properties via a central site of action, possibly on dopamine receptors in the chemoreceptor trigger zone (Peng, 1963). Domperidone is reported to be a new specific anti-nauseant compound (Laduron & Leysen, 1979) which also increases the rate of gastric emptying. It is a potent dopamine antagonist *in vitro* but does not appear to enter the brain in significant amounts after peripheral administration (Laduron & Leysen, 1979). This has led to the suggestion that domperidone may exert its anti-emetic action through interaction with dopamine receptors in the gastrointestinal tract (Van Nueten & Janssen, 1978). In man, one of the effects of domperidone that may be involved in its anti-emetic action is that of increasing lower oesophageal sphincter pressure, thus preventing gastro-oesophageal reflux. In a normal individual, a pressure gradient exists across the junction from the abdominal cavity, which has a positive pressure, to the thoracic cavity, which has a negative pressure (Fisher & Cohen, 1978). It is, therefore, necessary to postulate an anti-reflux mechanism to prevent backward movement of chyme into the oesophagus.

Specific dopamine receptors have been identified in the lower oesophageal sphincter of the opossum

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(Mukhopadhyay & Weisbrodt, 1974) and dopamine has been shown in this species to produce a relaxation of the lower oesophageal sphincter *in vivo* which could be blocked by haloperidol but not phentolamine or propranolol (Rattan & Goyal, 1976). Recently, it has been reported that the relaxation of the guinea-pig isolated stomach produced by dopamine was blocked by domperidone (Van Nueten, Ennis, Helsen, Laduron & Janssen, 1978). It seemed of interest, therefore, to investigate the effect of dopamine on the lower oesophageal sphincter of the guinea-pig *in vitro*, first to detect whether dopamine had any effect in this region and, secondly, to characterize its mechanism of action.

Methods

Guinea-pigs of either sex and weighing between 300 and 500 g were killed by a blow to the head and exsanguinated. The abdomen was opened and the stomach, together with 3 cm of oesophagus, was removed and immersed in warm, aerated Krebs-Henseleit solution.

The stomach contents were removed by washing with Krebs-Henseleit solution and an incision made in the stomach in the mid-corporal region bisecting it. The outer skeletal muscle coat of the oesophagus was removed according to the method of Bailey (1965). The proximal 2 cm of the oesophagus was discarded, leaving 1 cm of oesophageal tissue including the junctional area. Two diametrically-opposed longitudinal incisions were made in the oesophagus and were continued into the corpus of the stomach; further cuts at a 45° angle to the first two produced two identical strips 3 to 4 mm in width, consisting of oesophageal muscle, junctional tissue and a small amount of gastric muscle, which was used to anchor the tissue to the tissue pillar.

The strips were set up in 20 ml organ baths containing Krebs-Henseleit solution, maintained at 37°C and aerated with 5% CO_2 and 95% O_2 under a 1 g resting tension. Isometric tension changes were measured by means of 2 oz Ether transducers connected to single channel Rikadenki (model B-104) potentiometric recorders.

The strips were allowed to equilibrate for at least 1 h. All drug additions were made in volumes of less than 0.5 ml. Concentration-effect curves to agonist drugs were obtained with a 10 min cycle, leaving the drug in contact with the tissue until a maximum effect had developed. A concentration producing 80% of the maximum response (EC_{80}) was selected and repeated until the response was reproducible. The effect of various concentrations of antagonist drug on these responses was investigated for each agonist in turn and a pA_2 value determined by a method described by Fozard, Mobarok Ali & Newgrosh (1979) based on that of Schild (1947). The molar concentration of antagonist which reduced the response to twice the ED_{50} of agonist to that of the ED_{50} in the absence of antagonist was determined. This was expressed as a negative logarithm. These pA_2 values do not imply any particular mechanism of antagonism.

Histological examination of the strips was performed on longitudinally cut sections, 14 μm thick. Alternate sections were taken for histological examination using trichrome staining (Drury & Wallington, 1967) and fluorescence histochemistry (Spriggs, Lever, Rees & Graham, 1966).

Drugs used were: clonidine hydrochloride (Boehringer Ingelheim), cocaine hydrochloride (May & Baker), desmethylimipramine hydrochloride (Ciba-Giegy), domperidone (Janssen Pharmaceutica), dopamine hydrochloride (Koch-Light Chemicals), haloperidol (Haldol) (Janssen Pharmaceutica), 5-hydroxytryptamine creatinine sulphate (Koch-Light Chemicals), isoprenaline sulphate (Koch-Light Chemicals), metoclopramide (Beecham Pharmaceuticals), methysergide bimaleate (Sandoz), prazosin hydrochloride (Pfizer), propranolol hydrochloride (Inderal) (ICI Pharmaceuticals), reserpine (B.D.H.), spiroperidol (Spiperone) (Janssen Pharmaceutica), tyramine hydrochloride (B.D.H.). Solution of catecholamines were prepared daily and contained 0.1% ascorbic acid. Stock solutions of spiroperidol and domperidone were prepared in 1.0 M tartaric acid and diluted with 0.9% w/v NaCl solution (saline). Stock solutions of pimozide were prepared by dissolving the drug in 3 drops of glacial acetic acid and 3 drops absolute ethanol; the required volume was made up with hot 5% glucose solution and dilutions prepared with saline. All other drugs were prepared in saline. Concentrations are given as molar. Statistical significance of differences between two groups of results was determined by the Mann-Whitney U Test (2-tailed).

Results

Effect of agonist drugs

Phenylephrine, over the range 5×10^{-7} M to 5×10^{-5} M, produced a concentration-related contraction of the strips, whilst isoprenaline (5×10^{-7} M to 2×10^{-5} M) produced a relaxation of the strips (Figure 1b and c). Dopamine, in concentrations of 10^{-6} M to 10^{-4} M, produced a concentration-related relaxation of the strips (Figure 1a) with a similar maximum response to that of isoprenaline. Noradrenaline, 10^{-6} M to 2×10^{-5} M, produced a biphasic response with a relaxation preceding a contraction, whilst clonidine, in concentrations up to 10^{-5} M, had no effect on the muscle strips. Acetylcholine

(10^{-8} to 10^{-5} M) produced a contraction of the muscle strips.

Effect of antagonist drugs

An EC_{80} was selected from the concentration-effect curves for each agonist. These doses were repeated in the presence of single doses of a series of antagonists which had previously been shown to be effective on guinea-pig ileum against the agonists used in this study (Ennis, Janssen, Schnieden & Cox, 1979). The contraction produced by 5×10^{-6} M phenylephrine was completely inhibited by 10^{-6} M phentolamine. This concentration of phentolamine had no effect on the relaxation produced by isoprenaline (10^{-5} M) but partially antagonized the relaxation produced by dopamine (6×10^{-5} M) by approximately 50%. Propranolol (10^{-6} M) had no effect on the contraction produced by phenylephrine (5×10^{-6} M) but completely antagonized the relaxation produced by 10^{-5} M isoprenaline (Figure 2). In the presence of propranolol, the relaxation produced by dopamine was reduced by approximately 25%. This concentration of dopamine was then repeated in the presence of phentolamine (10^{-6} M) and propranolol (10^{-6} M) added simultaneously to the organ bath. The relaxation was completely antagonized. 5-Hydroxytryptamine produced a contraction of the strip in a concentration of 3×10^{-7} M which was unaffected by the presence of domperidone (4×10^{-7} M) but abolished by methysergide (3×10^{-6} M). The contraction produced by acetylcholine (10^{-6} M) was antagonised by atropine (10^{-8} M).

Since these results were of a purely qualitative nature, more quantitative experiments were performed with a range of antagonist concentrations to obtain a pA_2 value for the antagonist against each agonist in turn. The pA_2 values are shown in Table 1. The pA_2 values for phentolamine were determined in the presence of propranolol and pA_2 values for propranolol in the presence of phentolamine.

It can be seen from Table 1 that phentolamine blocked phenylephrine and dopamine but not isoprenaline whilst propranolol antagonized isoprenaline and dopamine but not phenylephrine. Prazosin

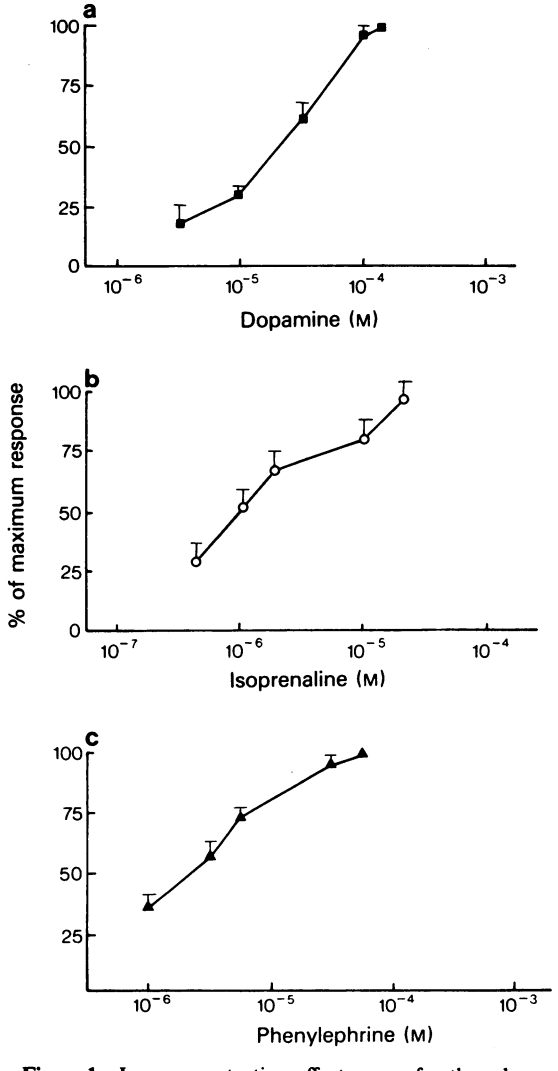

Figure 1 Log concentration-effect curves for the relaxation induced by dopamine (a), isoprenaline (b) and the contraction induced by phenylephrine (c) of longitudinal muscle strips of the guinea-pig gastro-oesophageal junction. Each point represents the mean response and the vertical bar the s.e. mean of 6 observations.

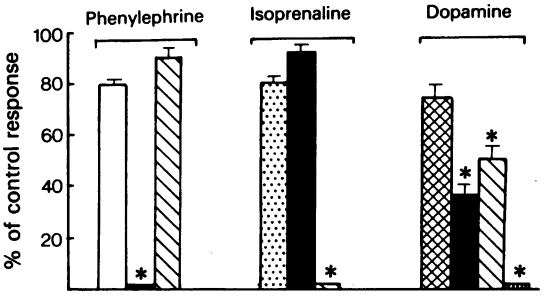

Figure 2 Effect of 10^{-6} M phentolamine alone (closed columns), 10^{-6} M propranolol alone (hatched columns) and both antagonists together (striped column) on EC_{80} responses to 5×10^{-6} M phenylephrine (open column), 10^{-5} M isoprenaline (stippled column) and 6×10^{-5} dopamine (cross hatched column). Each column represents the mean and the vertical bar the s.e. mean of 6 observations. *Significant antagonism, $P < 0.01$.

was found to be the most potent antagonist of the contraction produced by phenylephrine and of the relaxation produced by dopamine although the pA_2 value against phenylephrine was significantly higher than that for dopamine. Similarly spiroperidol was found to be a potent antagonist of phenylephrine and dopamine but again the pA_2 value against phenylephrine was significantly higher than against dopamine.

Domperidone and haloperidol were of similar potency as antagonists of phenylephrine and dopamine whilst pimozide and metoclopramide were found to be ineffective against all three agonists in concentrations up to 10^{-6} M and 5×10^{-5} M respectively. With the exception of propranolol none of the antagonists modified the relaxation induced by isoprenaline.

Indirectly acting sympathomimetics

Tyramine produced a concentration-related relaxation of the gastro-oesophageal junction strips over the range of 3×10^{-5} M to 2.5×10^{-4} M. The maximum effect obtained to tyramine was similar to that obtained with dopamine. There was no evidence of tachyphylaxis after repeated administration of tyramine. The relaxation produced by tyramine was blocked by domperidone with a pA_2 value of 6.2 ± 0.1. This value is significantly lower than that for dopamine ($P < 0.01$).

Effect of uptake inhibitors

Desmethylimipramine (3×10^{-7} M) almost completely abolished the concentration-effect curve to tyramine. This concentration of desmethylimipramine had very little effect on dopamine although increasing the concentration to 10^{-6} M produced a flattening of the concentration effect curve to dopamine (Figure 3a).

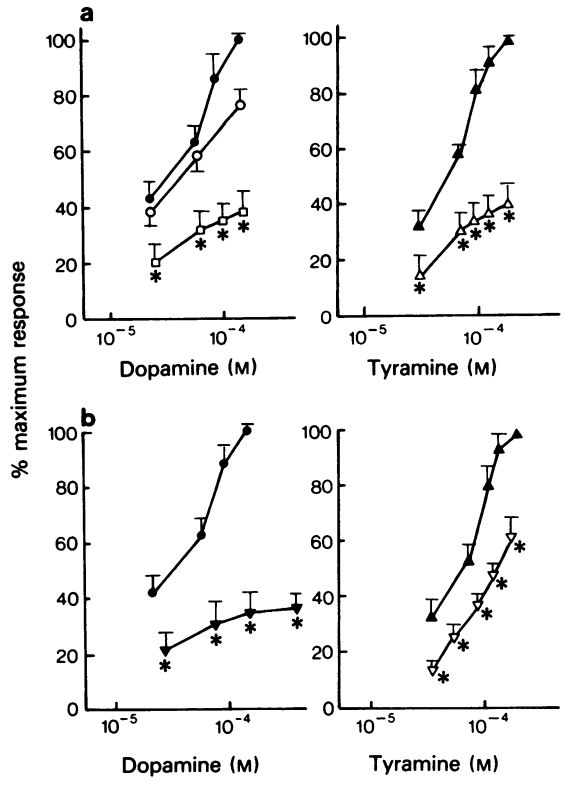

Figure 3 Log concentration-effect curves obtained on longitudinal muscle strips of the guinea-pig gastro-oesophageal junction for dopamine alone (●) and in the presence of (a) desmethylimipramine 3×10^{-7} M (○) or 10^{-6} M (□) and (b) cocaine 6×10^{-6} M (▼) and for tyramine alone (▲) and in the presence of (a) desmethylimipramine 3×10^{-7} M (△) and (b) cocaine 6×10^{-6} M (▽). Each point represents the mean and the vertical bar the s.e. mean of 6 observations. *Significant antagonism, $P < 0.01$.

Table 1 pA_2 values for the antagonism of the contraction produced by phenylephrine and the relaxation produced by either isoprenaline or dopamine of the guinea-pig isolated gastro-oesophageal junction

	pA_2 values ± s.e.		
Antagonist	*Phenylephrine*	*Isoprenaline*	*Dopamine*
Phentolamine	7.6 ± 0.3	No effect	7.5 ± 0.1
Propranolol	No effect	7.2 ± 0.3	7.7 ± 0.1
Prazosin	*9.7 ± 0.1	No effect	8.6 ± 0.2
Spiroperidol	*9.5 ± 0.1	No effect	8.6 ± 0.2
Domperidone	6.3 ± 0.2	No effect	6.6 ± 0.1
Haloperidol	6.7 ± 0.1	No effect	6.1 ± 0.1
Pimozide	No effect	No effect	No effect
Metoclopramide	No effect	No effect	No effect

* denotes pA_2 value for phenylephrine significantly different from that for dopamine, $P < 0.01$.

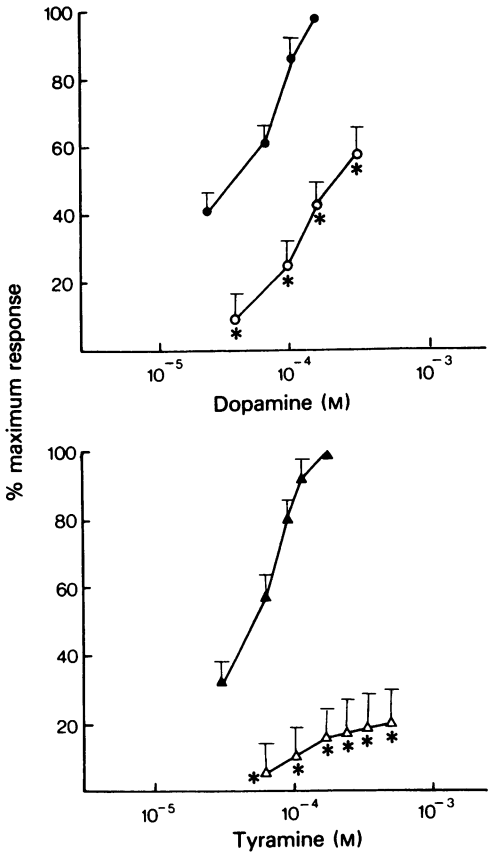

Figure 4 Log concentration-effect curves for dopamine (●○) and tyramine (▲△) on longitudinal muscle strips of control (closed symbols) and reserpine pretreated (open symbols) guinea-pig gastro-oesophageal junction strips. Each point represents the mean and the vertical bar the s.e. mean of 6 observations. *Significant antagonism, $P < 0.01$.

It was not possible to overcome the effect of desmethylimipramine by increasing the concentration of either dopamine or tyramine.

Cocaine (6×10^{-6} M) produced a shift to the right in the concentration-effect curve to tyramine with a ratio of 3.8 measured at the EC_{50} level. Cocaine (6×10^{-6} M) almost completely abolished the concentration-effect curve to dopamine (Fig. 3b).

The concentration of desmethylimipramine and cocaine used in this series of experiments potentiated the relaxant component of the response to noradrenaline.

Effect of depletion of noradrenaline stores

In gastro-oesophageal junction strips taken from guinea-pigs pretreated with reserpine 5 mg/kg, 16 h before they were killed it was found that the relaxation produced by tyramine was abolished whilst the concentration-effect curve to dopamine was shifted to the right with a ratio of 2.5 measured at the EC_{50} level (Figure 4). The size of the maximum response to dopamine was not reduced in the same way as the maximum response to tyramine.

Histological examination of the gastro-oesophageal junction strips

It was found that the smooth muscle of longitudinal strip preparations of the gastro-oesophageal junction was composed primarily of longitudinal fibres of muscularis mucosa. It was possible to identify the presence of the sphincter region in this preparation because transition from striated to smooth muscle in the muscula externa that remained after most of this layer had been stripped away could still be seen.

Fluorescence histochemistry revealed that there was a sparse catecholaminergic innervation present in the preparation other than that associated with blood vessels. Because of the low density of the innervation it was not possible to identify the nature of the catecholamine in the tissue.

Discussion

The effects of dopamine on gastro-oesophageal junction tissue taken from guinea-pigs has been examined to determine if dopamine could be acting via specific dopamine receptors at this site. Initially, as this is a sphincter region, spirally cut muscle strips were compared with longitudinal strips. In all cases the response of both types of strip were identical irrespective of the agonist or antagonist used, confirming the observations of Bailey (1965). However, since only one spiral strip could be obtained from each animal and since the spiral strips developed less spontaneous tone than the longitudinal strips, it was decided to continue the quantitative aspects of the study with the longitudinal strips.

Tsai, Langer & Trendelenburg (1967) described two components in the mechanism of action of dopamine on smooth muscle, a direct action on post-synaptic dopamine or adrenoceptors and an indirect action via the release of noradrenaline.

The mechanism of action of dopamine on longitudinal muscle strips of the guinea-pig gastro-oesophageal junction was initially investigated by comparing the response obtained to dopamine with the type of response obtained to agonists reported to be specific for certain types of receptor. Phenylephrine, which interacts preferentially with α_1-adrenoceptors (Starke, 1972), produced a contraction of the strips which was sensitive to blockade by phentolamine and

the α_1-adrenoceptor antagonist prazosin (Cambridge, Davey & Massingham, 1977). Clonidine which acts specifically on α_2-adrenoceptors (Starke, Montel, Gayk & Merker, 1974) was ineffective, thus the α-adrenoceptors present in the gastro-oesophageal junction strips appear to resemble α_1-adrenoceptors. The response of the tissue to stimulation of these receptors was a contraction which suggests that the receptors were excitatory.

Isoprenaline on the other hand produced a relaxation of the muscle strips which was blocked only by propranolol, a β-adrenoceptor antagonist. Thus the β-adrenoceptors in this tissue appear to mediate inhibition. Dopamine produced a relaxation which was blocked partly by α- and partly by β-adrenoceptor antagonists. Thus the α-adrenoceptors activated by dopamine appear to be purely inhibitory. Haffner, Liåvag & Setekleiv (1969) also found a mixture of excitatory and inhibitory α-adrenoceptors in human stomach and pylorus.

From the quantitative experiments using the antagonists it was possible to obtain a relative order of potency of the compounds as antagonists of the contraction produced by phenylephrine and the relaxation induced by dopamine. For both types of response this was found to be prazosin > spiroperidol > phentolamine > domperidone > haloperidol. Pimozide and metoclopramide were not effective antagonists of either phenylephrine or dopamine. Leysen, Gommeren & Laduron (1978) have compared the relative potency of a series of dopamine antagonists in displacing tritiated haloperidol from specific binding sites in rat striatal homogenates. The order of potency obtained by this method for the compounds used in the present study was spiroperidol > domperidone > pimozide > haloperidol > metoclopramide. However, in our study pimozide and metoclopramide were ineffective, a finding which suggests that the compounds were not acting on a conventional dopamine receptor. It seemed possible, therefore that the dopamine-induced relaxation of the gastro-oesophageal junction strips was due to an interaction with both α- and β-adrenoceptors. The α-adrenoceptor involved may more closely resemble α_1- than α_2-type since clonidine was ineffective. However, this does not explain why phenylephrine produced a contraction of the gastro-oesophageal junction strips whilst dopamine induced a relaxation. Thus the two compounds cannot have been acting on the same population of α-adrenoceptors. Although the relative order of antagonist potency was the same for both dopamine and phenylephrine, there were some quantitative differences in the absolute pA_2 values. Thus prazosin, spiroperidol and domperidone were more potent as antagonists of phenylephrine. This could perhaps be explained by postulating that a component of the effect of dopamine is indirect and that the released transmitter was less well antagonized than exogenously applied drug.

It has been suggested that 5-hydroxytryptamine may be involved in the modulation of gastric activity (Bülbring & Gershon, 1967). Therefore an examination of the effects of this compound was included in this study. It was found that 5-hydroxytryptamine induced a contraction of the strips which was sensitive to blockade by methysergide but not by domperidone, indicating a lack of any 5-hydroxytryptamine antagonism in the mode of action of domperidone. The possibility arises that dopamine may not act only by an interaction with post-junctional receptors but also through the release of noradrenaline. To investigate this possibility, tyramine, a compound known to act as an indirect sympathomimetic (Iversen, 1967), was included in the study for comparative purposes. Tyramine was shown to produce similar responses to dopamine although the concentration of tyramine required to produce this effect was slightly higher. Surprisingly, there was no obvious tachyphylaxis to tyramine, as is usually observed with an indirectly acting compound, suggesting that tyramine may not be acting wholly through the release of noradrenaline. Domperidone, which antagonized the relaxation produced by dopamine on the strips, also antagonized the response to tyramine although the pA_2 value for the antagonism of tyramine was significantly less than that for dopamine which suggests that the mechanisms of action of dopamine and tyramine were not identical. This would be expected if the indirect component in the action of tyramine was greater than that of dopamine.

To investigate further the mechanism of action of tyramine and dopamine the effects of two noradrenaline uptake inhibitors were investigated. Desmethylimipramine (3×10^{-7} M) has been shown to inhibit noradrenaline uptake into sympathetic nerves in rat iris (Farnebo & Hamberger, 1971). This concentration of desmethylimipramine reduced the size of the response to tyramine over the whole of the concentration range, indicating that tyramine was acting as predicted, predominantly as an indirect sympathomimetic. However, this concentration of desmethylimipramine had very little effect on the size of the relaxation induced by dopamine although a higher concentration of 10^{-6} M did produce a flattening of the dopamine concentration-effect curve. It has been suggested that this higher concentration of desmethylimipramine has post-junctional α-adrenoceptor blocking activity (McCulloch & Story, 1972) which again indicates that dopamine may have been acting on α_1-adrenoceptors to produce the relaxation.

Cocaine has been shown to inhibit the uptake of noradrenaline into sympathetic nerves (Trendelenburg, 1972). In contrast to the results obtained with desmethylimipramine, cocaine (6×10^{-6} M) preferen-

tially antagonized the relaxation induced by dopamine; thus the common property of uptake inhibition cannot adequately explain these observations.

Reserpine-treatment emphasised the differences between tyramine and dopamine, since depletion of amine stores abolished the response to tyramine but only partially antagonized the response to dopamine. Although a fluorescence histological examination of the preparation revealed only a sparse adrenergic innervation, this must have been sufficient to mediate the effects of tyramine. Since smooth muscle elements were shown to be present in the preparation, the reserpine-insensitive component of the action of dopamine probably involves the stimulation of postjunctional receptors. However, the precise nature of these receptors remains to be determined.

In conclusion therefore, although dopamine produced a relaxation of the guinea-pig longitudinal muscle strip of the gastro-oesophageal junction, perhaps theoretically mimicking the *in vivo* situation, the evidence does not support mediation via a conventional dopamine receptor. It seemed more likely that dopamine was acting partly indirectly but mainly postsynaptically on a mixture of α- and β-adrenoceptors. The α-adrenoceptors appear to resemble closely, but may not be identical with α_1-adrenoceptors. Since there was no evidence for specific dopamine receptors some other mechanism of action must be responsible for the antagonism of dopamine by domperidone.

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