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# On the ability of domperidone to selectively inhibit catecholamine-induced relaxation of circular smooth muscle of guinea-pig stomach

H. A. SAHYOUN, B. COSTALL AND R. J. NAYLOR*

*Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP, U.K.*

The effects of noradrenaline and dopamine, and their interactions with α- and β-adrenoceptor antagonists and with domperidone, were studied on circular smooth muscle strips taken from the cardia, fundus, body and antrum of the guinea-pig stomach. Noradrenaline and dopamine caused relaxations of all tissues which were generally susceptible to antagonism by either propranolol or phentolamine in concentrations shown to antagonize the relaxations caused by isoprenaline or phenylephrine respectively. In addition, dopamine, in concentrations subthreshold for relaxation, caused contraction of the muscle strips which increased in intensity from the cardia to the antral region: these contractions were antagonized by phentolamine and yohimbine but were insensitive to prazosin: prazosin selectively inhibited the phenylephrine relaxations. With the exception of a modest reduction in the responses of the cardia to dopamine, all tissue responses to noradrenaline and dopamine were resistant to reserpine. Domperidone and haloperidol were found to selectively inhibit the phenylephrine- noradrenaline- and dopamine-induced relaxations of the stomach strips and to enhance the contractile component of dopamine's action: this ability of domperidone to facilitate a dopamine induced contraction, which was most marked in the body and antral regions, was prevented by phentolamine. It is thus concluded that domperidone antagonizes noradrenaline- and dopamine-induced relaxations at one adrenoceptor site having characteristics conistent with an $\alpha_1$-adrenoceptor type whilst failing to antagonize at a further dopamine-sensitive adrenoceptor site involved in contraction of circular smooth muscle of the stomach and having characteristics consistent with an $\alpha_2$-adrenoceptor.

Domperidone facilitates gastric emptying in animals and in man (De Schepper et al 1978; Reyntjens et al 1978; Van Neuten et al 1978). Because domperidone may be categorized with the neuroleptic drugs, the classical dopamine antagonists, it was originally considered that its gastrokinetic action might involve interaction with dopamine receptors in the upper gastrointestinal system. However, some observations now question this hypothesis, firstly, not all neuroleptic agents can antagonize a dopamine response in the gastrointestinal system (Cox & Ennis 1979), secondly, prazosin, an α-adrenoceptor blocking agent, can effectively antagonize such a dopamine response (Cox & Ennis 1979) and, thirdly, in other peripheral preparations, for example the aortic strip, domperidone is shown to exert α-antagonist activity (Ennis & Cox 1980). To see if these observations are relevant to the action(s) of domperidone on the stomach, we have selected four regions of circular smooth muscle of the guinea-pig stomach—cardia, fundus, body and antrum— for characterization of the dopamine and/or adrenoceptor mechanisms controlling the ability of that muscle to contract and relax.

* Correspondence.

## MATERIALS AND METHODS

Male Dunkin-Hartley guinea-pigs, 350–450 g, that had been starved overnight, were killed by cervical trans-section. The stomach was removed with approximately 2 cm of oesophagus and duodenum and placed in Krebs and Henseleit solution for dissection of smooth muscle strips from the cardia, fundus, body and antrum of the stomach. The strips were dissected in a plane suitable to investigate tension changes in the circular muscle layer. The mucosal layer was removed and the tissue bathed in 15 ml oxygenated (95% $O_2$; 5% $CO_2$) Krebs and Henseleit solution at 37 °C containing 100 mg litre$^{-1}$ ascorbic acid. Tension changes were detected by Grass tension transducers and the response area integrated (Illingworth & Naylor 1980) in addition to display on a multichannel Grass recorder. One gram tension was applied to the tissues which were allowed to equilibrate for 30–45 min before the addition of drugs. Contact time for noradrenaline, dopamine, isoprenaline and phenylephrine was 5 min. Example traces of relaxation and/or contraction responses to dopamine in each of the stomach strips are shown in Fig. 1 and the maximum changes in tension to be recorded from the 4 stomach strips using the 4 agonists are shown in Fig. 2. Antagonists were

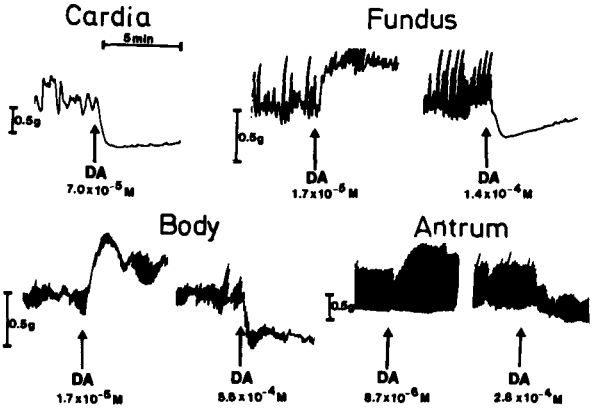


FIG. 1. Example traces of relaxation and/or contraction responses to dopamine (DA) in circular smooth muscle preparations taken from the cardia, fundus, body and antrum of guinea-pig stomach.

allowed a 20 min incubation period before the addition of agonists. Domperidone (Janssen) and phentolamine mesylate (Rogitine, Ciba) were used in the parenteral forms prepared by the manufacturers; (±)-propranolol hydrochloride (ICI), noradrenaline hydrogen tartrate (Hoechst), dopamine hydrochloride (Koch Light), isoprenaline sulphate (Riker), yohimbine hydrochloride (Sigma), prazosin hydrochloride (Pfizer) and phenylephrine hydrochloride (BDH) were prepared in distilled water, haloperidol (Janssen) and reserpine (BDH) in the minimum quantity of tartaric acid and *NN*-dimethylformamide respectively.

In all experiments appropriate solvent controls were performed. Significant differences in data obtained was assessed using the Mann-Whitney 'U' test.

## RESULTS

Noradrenaline ($10^{-7}$ – $10^{-5}$ M), dopamine ($10^{-5}$ – $10^{-3}$ M), isoprenaline ($4 \times 10^{-8}$ – $5 \times 10^{-5}$ M) and phenylephrine ($10^{-7}$ – $10^{-5}$ M) caused concentration-dependent relaxations of the circular smooth muscle taken as strips from the cardia, fundus, body and antrum of guinea-pig stomach. In addition, concentrations of dopamine subthreshold for relaxation ($10^{-6}$ – $10^{-5}$ M) caused contraction in the fundus, body and antral strips. A contractile response to noradrenaline was markedly less (Figs 3, 4).

Domperidone ($10^{-5}$ M) caused 10–40 fold shifts to the right in the concentration-response curves for relaxation to dopamine, noradrenaline and phenylephrine in all four tissues. The antagonism of relaxation by domperidone was accompanied by exaggerated contractile responses to dopamine in the fundus, body and antrum, and a contractile response to phenylephrine was revealed in the antral preparation (Figs 3, 4). Domperidone, $10^{-6}$ M (but not $10^{-7}$ M) also shifted the relaxation response curves to the right (approximate 3 fold shifts). At all concentrations, and in all tissues, domperidone failed to modify the relaxation caused by isoprenaline.

An attempt to characterize the sites of action of noradrenaline and dopamine and, consequently, domperidone analysed the effects of propranolol,

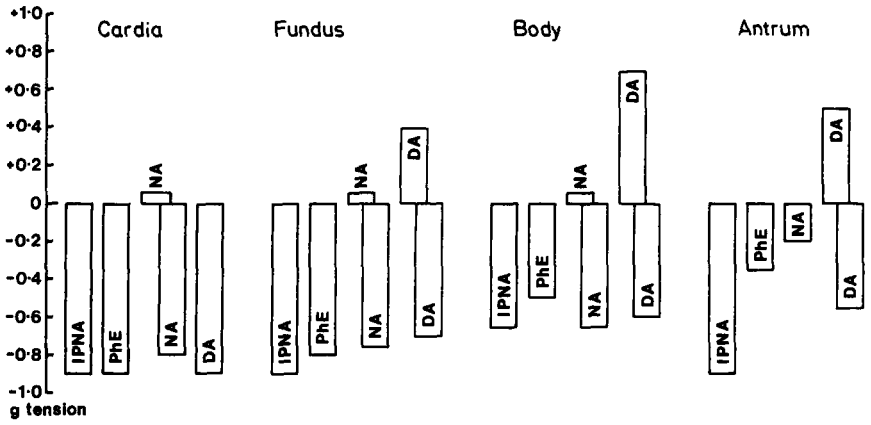


FIG. 2. Maximum tension changes (g) caused by isoprenaline (IPNA), phenylephrine (PhE), noradrenaline (NA) and dopamine (DA) at their maximally effective concentrations (see Figs 3, 4) on circular smooth muscle strips taken from the cardia, fundus, body and antral regions of guinea-pig stomach. The + values on the ordinate indicate contraction, the − values relaxation. n = 6. S.e.m.s <12%. The maximum 'response' is a product of the maximum tension change and the duration of this change, thus, response 'sizes' are determined in subsequent figures by integration of the 'area' of contraction or relaxation.

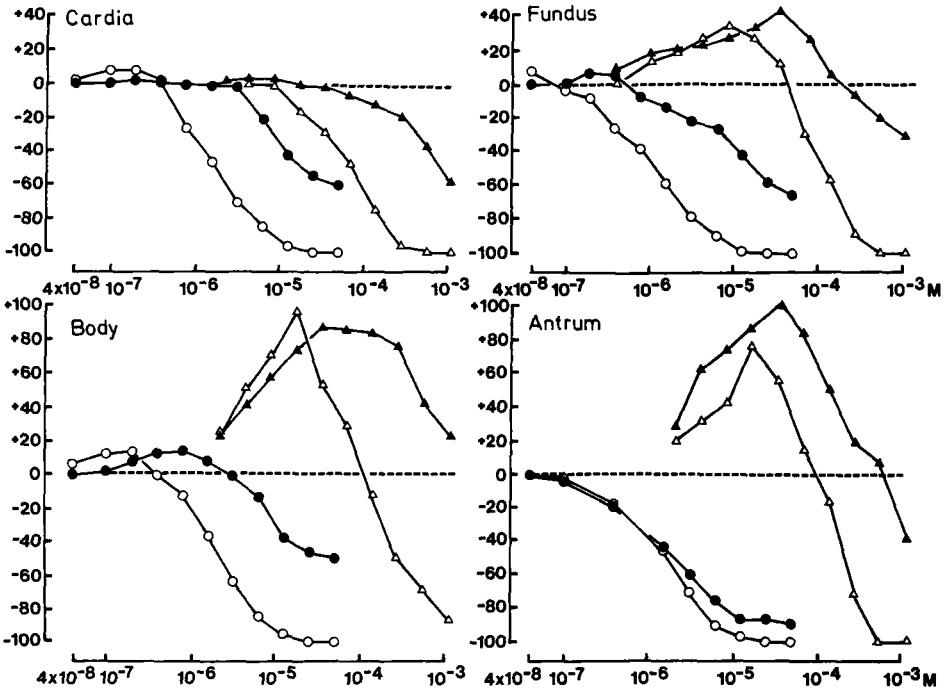


FIG. 3. Relationships between concentration and response for noradrenaline ○—○ and dopamine △—△ in circular smooth muscle preparations taken from the cardia, fundus, body and antrum of guinea-pig stomach. ●—● and ▼—▼ indicate shifts in the curves for noradrenaline and dopamine respectively in the presence of $10^{-5}$ M domperidone. The + values on the ordinate indicate contraction, the − values relaxation. The size of response was determined by integration of the area of response: in order to compare 'sizes' of contraction and relaxation responses, the size of the maximum relaxation response to each agonist was termed 100% and the sizes of all other relaxation and contraction responses determined as a percentage of this maximum. n = 6. S.e.m.s. <12%.

phentolamine and haloperidol on the marked relaxation responses to submaximal concentrations of isoprenaline ($3{\cdot}2 \times 10^{-7}$ M), phenylephrine ($8 \times 10^{-6}$ M), noradrenaline ($2 \times 10^{-6}$ M) and dopamine ($1{\cdot}4 \times 10^{-4}$ M). Preliminary studies established a suitable concentration of propranolol to antagonize at β-adrenoceptors as one inhibiting the isoprenaline response. Propranolol at $10^{-8}$ M was ineffective in reducing the isoprenaline relaxation, $10^{-7}$ M caused an approximate 50% reduction and $5 \times 10^{-7}$ M markedly reduced the response in all tissues. In all tissues propranolol, $5 \times 10^{-7}$ M, failed to reduce the phenylephrine-relaxations. It also failed to reduce the relaxations in the cardia caused by noradrenaline and dopamine while it significantly decreased them in the fundus and body strips. However, in the antral preparations the dopamine relaxation could not be antagonized by propranolol although the noradrenaline relaxations were markedly reduced (Fig. 5B).

A suitable concentration of phentolamine to antagonize at α-adrenoceptors was assessed as one capable of antagonizing the phenylephrine relaxation. Phentolamine at $10^{-8}$ M was ineffective, $10^{-7}$ M caused an approximate 20% reduction whilst $10^{-6}$ M markedly reduced or abolished the phenylephrine-induced relaxations in all four tissues. Phentolamine ($10^{-6}$ M) similarly caused marked reductions in both the noradrenaline and dopamine relaxations in the cardia, fundus and body whilst only the dopamine relaxation was reduced in the antral strips (Fig. 5C).

A combined treatment of propranolol ($5 \times 10^{-7}$ M) and phentolamine ($10^{-6}$ M) markedly reduced or abolished the relaxation responses to all four agonists in all four tissues (Fig. 5G).

The most significant effect of a combined domperidone ($10^{-5}$ M) plus phentolamine ($10^{-6}$ M) treatment was to markedly reduce the contractions caused by the dopamine plus domperidone regime in the fundus, body and antrum. Indeed, in the fundus and antrum the contractions were reversed to relaxations. Similarly, the noradrenaline-induced

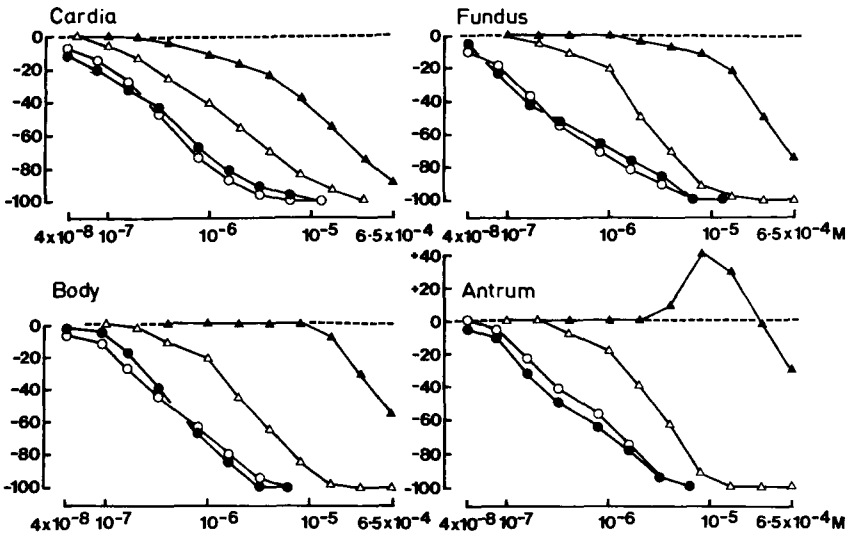


FIG. 4. Relationships between concentration and response for isoprenaline ○—○ and phenylephrine △—△ in circular smooth muscle preparations taken from the cardia, fundus, body and antrum of guinea-pig stomach. ●—● and ▼—▼ indicate shifts in the curves for isoprenaline and phenylephrine respectively in the presence of $10^{-5}$ M domperidone. Other details as Fig. 3.

relaxations observed after domperidone treatment were enhanced by phentolamine in the three tissues. Only in the cardia, where contractions were not observed to the agonists alone or agonist plus domperidone, did the combined phentolamine plus domperidone treatment fail to have an additional effect, with the sole exception that the phenylephrine relaxation was further reduced (Fig. 5E).

Haloperidol ($10^{-7}$ M) reduced the relaxation responses to phenylphrine, noradrenaline and dopamine in the cardia by 20–35%, these reductions being increased to 65–80% at $10^{-6}$ M and to approximately 90% at $10^{-5}$ M (Fig. 5F). The other three tissues were slightly more sensitive, showing the marked reductions at $10^{-6}$ M haloperidol against phenylephrine, dopamine and noradrenaline relaxations. Furthermore, in the body and antrum the relaxations were converted to contractions (Fig. 5F). These results are similar to those obtained with domperidone (Fig. 5D). At all concentrations and in all tissues haloperidol did not modify the isoprenaline relaxation.

In a more detailed analysis of the dopamine-induced contractions of circular smooth muscle of the body strip, propranolol ($5 \times 10^{-7}$ M) was routinely included in the Krebs and Henseleit solution to preclude relaxation effects via β-adrenoceptors. Yohimbine was found to antagonize the dopamine contraction in a concentration-dependent manner, the parallel shift of the response curves to the right suggesting a competitive antagonism. At the concentrations used to antagonize the dopamine response, yohimbine was ineffective in antagonizing phenylephrine-induced relaxations. Conversely, prazosin was ineffective in modifying the dopamine contractions in concentrations that shifted the phenylephrine relaxation curves to the right (Fig. 6).

In an examination of the effects of a reserpine pretreatment on the effects obtained to noradrenaline and dopamine, guinea-pigs were given reserpine 5 mg $kg^{-1}$ i.p. 24 h before death. This regime was established as causing a reduction in the tyramine response of at least 70% in tissues taken from such animals. With the exception of a very modest reduction in the relaxation responses to dopamine in the cardia, all tissue responses to noradrenaline (cardia, fundus, body, antrum) were resistant to change by reserpine (unpublished data).

## DISCUSSION

The first important observation of the present study is that dopamine can cause a marked contraction of the circular smooth muscle of the guinea-pig stomach in addition to the documented ability to cause relaxation (Van Neuten et al 1978); noradrenaline has a similar biphasic action but the contraction phase is relatively less marked. Isoprenaline and phenylephrine do not possess this dual action, but cause relaxation responses only. Komissarov &

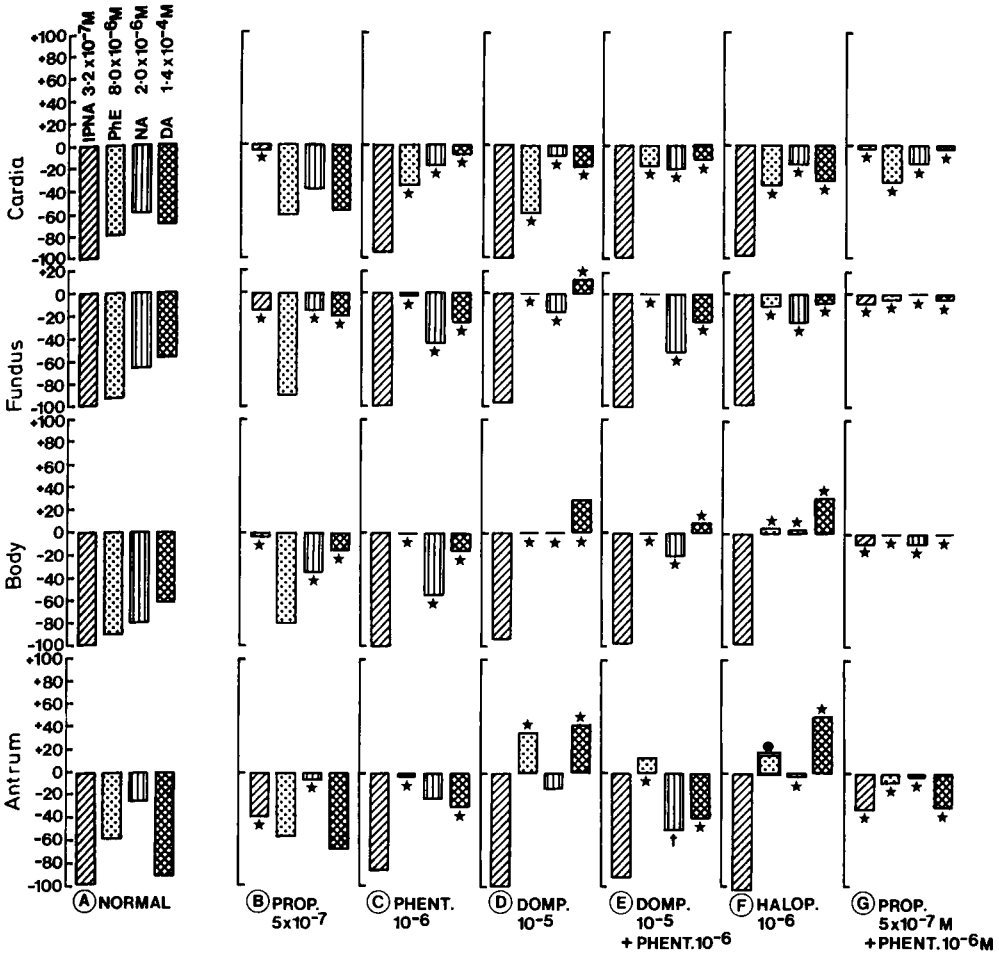


FIG. 5. Abilities of submaximal concentrations of isoprenaline (IPNA, ▨), phenylephrine (PhE, ▩), noradrenaline (NA, ▥) and dopamine (DA, ▩) to cause relaxation of normal tissue taken from the cardia, fundus, body or antrum of the guinea-pig stomach (column A). Columns B-G indicates the changes in the relaxation responses caused by treatment with propranolol (Prop.), phentolamine (Phent.), domperidone (Domp.), a combination of domperidone and phentolamine, haloperidol (Halop.), and a combination of propranolol and phentolamine. The + values on the ordinate indicate contraction, the − values relaxation. The size of response (relaxation or contraction) was determined by integration of the area of response and is presented as a % of the maximum relaxation obtained with isoprenaline. n = 6. S.e.m.s <12%, * indicates a significant reduction in response, † a significant increase ($P < 0{\cdot}05$, Mann-Whitney 'U' test).

Reutskaya (1978) have also reported that small doses of noradrenaline and dopamine cause contraction of the rat fundus, but in the present studies we extend these observations to indicate differences both between noradrenaline and dopamine and between the responses of different stomach regions with respect to a contractile-relaxant potential.

On the bases of a number of observations, we interpret data obtained for dopamine in the present studies as being largely independent of noradrenaline release. Thus, (a) some of the responses to dopamine are qualitatively and/or quantitatively different from responses to noradrenaline, (b) reserpine pretreatment does not modify the dopamine effects in the fundus, body or antrum and causes only a modest reduction in dopamine response from the cardia. We accept that the actual site of action of dopamine, neuronal and/or on smooth muscle, remains to be investigated.

The ability of dopamine to cause contraction of the circular smooth muscle of the stomach increased from the cardia to the fundus and reached a maximum in the body and antrum. In contrast, there was little variation in the ability of noradrenaline to cause contraction of the preparations from the cardia, fundus or body regions of the stomach and,

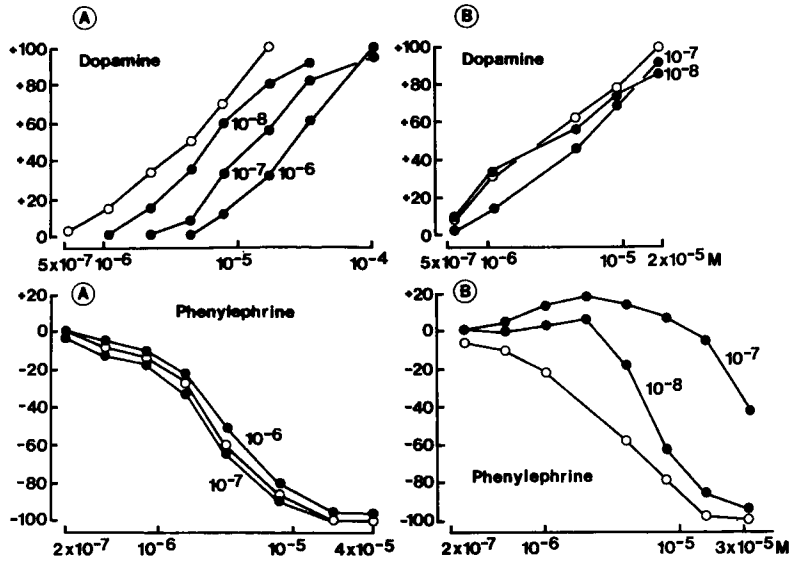


FIG. 6. Effects of A. yohimbine and B. prazosin (M concentrations indicated on graphs, ●—●) on the contraction/relaxation responses of the body strip to dopamine (○—○) and phenylephrine. The + values on the ordinate indicate contraction, the − values relaxation. The size of response was determined by integration of the area of response: values are expressed as a percentage of the maximum contraction to dopamine (control response, ○—○) or maximum relaxation to phenylephrine (again, control response, ○—○). n = 6. S.e.m.s. <12%.

indeed, noradrenaline failed to cause contraction of the antral tissue. Furthermore, even within the body region, where the ability of noradrenaline to cause contraction of the smooth muscle was most marked, the size of the response was still less than 50 percent that induced by dopamine. Initial attempts to characterize the nature of the adrenoceptors involved in the contractile phase of dopamine's action indicated that phentolamine completely antagonized the powerful dopamine-induced contractions observed after domperidone treatment. Phentolamine possesses both $\alpha_1$- and $\alpha_2$-adrenoceptor antagonist action in some tissues and more detailed characterization of the α-adrenoceptor mechanism mediating the contractile effects of dopamine in the body strip utilized prazosin and yohimbine, agents having preferential $\alpha_1$- and $\alpha_2$-adrenoceptor antagonist action, respectively. Yohimbine competitively antagonized the dopamine contractions at concentrations that failed to attenuate the phenylephrine relaxations; in contrast, prazosin, in concentrations shown to competitively antagonize phenylephrine relaxations, was ineffective in antagonizing the dopamine contraction. We therefore conclude that the contractile effect of dopamine is mediated at an $\alpha_2$-type adrenoceptor.

Experiments aimed at a characterization of the adrenoceptor mechanisms involved with the relaxation phase of the response to noradrenaline and dopamine revealed an interaction at *both* α and β sites. Thus, both phenylephrine and isoprenaline were shown to cause a relaxation of the muscle preparations from the cardia, fundus, body and antrum, and these effects were markedly reduced or abolished respectively by phentolamine and propranolol. The relaxations of the tissues caused by noradrenaline and dopamine were also reduced by both phentolamine and propranolol. These studies revealed that the α- and β-receptor population may vary from area to area. Thus, the abilities of noradrenaline and dopamine to cause relaxation of the cardia were preferentially reduced by phentolamine, but both phentolamine and propranolol antagonized the relaxation responses of the fundus and body whilst, in the antrum, noradrenaline appeared to act exclusively via a propranolol-sensitive site with dopamine acting on a phentolamine-sensitive site. Hence, we conclude that within the stomach noradrenaline and dopamine have a potential to cause contraction of the circular smooth muscle via phentolamine/yohimbine sensitive sites (ie via $\alpha_2$-adrenoceptors) and that these two catecholamines and phenylephrine mediate a relaxation at either phentolamine/prazosin sensitive sites (ie via α-adrenoceptors) *or* propranolol sensitive sites (ie via β-adrenoceptors).

The second important observation of the present study relates to the action of domperidone and haloperidol in antagonizing the abilities of dopamine to relax the stomach tissues whilst failing to antagonize the contractile effects. Firstly, this would lead to the immediate conclusion that, in the guinea-pig stomach, the adrenoceptors mediating the dopamine contraction and relaxation are not the same, with the neuroleptic agents exerting a selective antagonism at the latter. Secondly, and the critical question, 'is this dopamine-neuroleptic interaction in the stomach mediated at a dopamine receptor?' must, on available data, be answered in the negative. Thus, domperidone and haloperidol antagonized noradrenaline- and phenylephrine-relaxations as effectively as those induced by dopamine. Notwithstanding that domperidone and haloperidol exert specific dopamine antagonist action in cerebral dopamine systems (Costall et al 1979) we would conclude, on the basis of the above receptor characterization in the stomach, that domperidone and haloperidol antagonize dopamine noradrenaline- and phenylephrine-induced relaxations at an $\alpha_1$-adrenoceptor; there is no evidence for an action on specific dopamine receptors. In an analysis of the action between dopamine and the neuroleptic agents in the associated gastro-oesophageal junction, Cox & Ennis (1980) have similarly concluded that neuroleptic agents, including domperidone, antagonize at $\alpha$-adrenoceptors.

Finally, and with particular respect to domperidone, could these in vitro results have any relevance to our understanding of drug action in vivo? Both agonists and antagonists were generally required in micromolar concentrations to cause the described changes, which may shed doubt on a possible physiological-pharmacological relevance. However, in the guinea-pig intact and isolated stomach preparation, domperidone has recently been shown to be equieffective as an antagonist of phenylephrine- and dopamine-induced relaxations (Schuurkes & Van Neuten 1981). Prazosin was also an effective antagonist of the phenylephrine- and dopamine-induced relaxations but there remained a component of the dopamine relaxation resistant to prazosin antagonism. The authors inferred the presence of a 'dopamine-specific' receptor in addition to the $\alpha_1$ mechanism. Thus, if the obvious limits to which in vitro data may be related to in vivo action is accepted, then the analysis of the observed contraction-relaxation responses to domperidone provide a rationale for further investigations of domperidone's action on catecholamine systems in vivo.

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