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EFFECTS OF METOCLOPRAMIDE AND ISOPRENALINE IN THE RAT VAS DEFERENS; INTERACTIONS WITH α-ADRENOCEPTORS

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1 Metoclopramide (2.8 to 280 μM) augmented contractions of rat vas deferens preparations induced by field stimulation (6 Hz for 1s). This effect was antagonized by phentolamine (0.1 μM). Metoclopramide (2.8 to 280 μM) did not affect phenylephrine-induced contractions.

2 Metoclopramide (2.8 to 280 μM) antagonized the inhibitory effects of clonidine on the contractions induced by field stimulation, but not the inhibitory effects of purine nucleosides.

3 From these results it is concluded that metoclopramide (2.8 to 280 μM) is a presynaptic α-adrenoceptor antagonist in the rat vas deferens.

4 Following β-adrenoceptor blockade with (±)-propranolol (3.3 μM), (−)-isoprenaline (0.47 to 14 μM) inhibited responses to field stimulation but not to phenylephrine. These propranolol-resistant effects of isoprenaline were antagonized by metoclopramide (2.8 to 280 μM) and by phentolamine (0.1 to 10 μM), indicating that isoprenaline may stimulate presynaptic α-adrenoceptors in this preparation.

Introduction

Metoclopramide is an antagonist of apomorphine-induced vomiting in dogs (Justin-Besançon & Laville, 1964) and increases dopamine turnover in mouse brain (Peringer, Jenner, Donaldson, Marsden & Miller, 1976; Elliott, Jenner, Huizing, Marsden & Miller, 1977). Metoclopramide has been recently claimed to be a specific antagonist of dopamine-induced vasodilatation in the dog kidney (Kohli, Volkman, Glock & Goldberg, 1978) and of dopamine-induced inhibition of [^{3}H]-noradrenaline release in rabbit ear artery (Hope, McCulloch, Rand & Story, 1978). However, metoclopramide does not antagonize the dopamine-induced elevation of cyclic adenosine 3',5'-monophosphate (cyclic AMP) in rat striatum (Peringer *et al.*, 1976) and lacks antipsychotic activity in man (Berenstein & Bles, 1965; Nakra, Bond & Lader, 1975). These findings may be explained if two, or more, sub-groups of the dopamine receptor exist (Goldberg, Kohli, Kotake & Volkman, 1978; Kebabian & Calne, 1979).

In view of the close similarity between some forms of dopamine receptor and α-adrenoceptors (Gothert, Lox & Rieckesmann, 1977), the effects of metoclopramide on clonidine-induced inhibition of the response of the rat vas deferens to field stimulation have been examined. The inhibitory effects of clonidine in this tissue have been characterized as being mediated via presynaptic α-adrenoceptors (Doxey, Smith & Walker, 1977; Drew, 1977), similar to the α_2-adrenoceptors reported in other tissues (Berthelson & Pettinger, 1977; Langer, 1977). During the course of this investigation it was discovered that isoprenaline, following β-adrenoceptor blockade by propranolol, may also interact with the presynaptic α-adrenoceptors.

Methods

Vasa deferentia from Sprague-Dawley rats (230 to 400 g) were set up in 10 ml isolated organ baths containing Tyrode solution maintained at 35 ± 1°C. The Tyrode solution of the following composition (mM): NaCl 137, KCl 2.7, $MgCl_2$ 1.1, $CaCl_2$ 1.8, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.5 and ascorbic acid 0.06 was gassed with 95% O_2 and 5% CO_2 and contained atropine (1.7 μM) to exclude the effects of cholinergic nerve stimulation. Resting tension was set at 200 to 300 mg and after a 30 min equilibration period, responses were recorded under isometric conditions, with Statham Gold cell strain gauges and a Grass 79D polygraph.

Field stimulation

Preparations (25 to 30 mm length) were set up between two platinum ring electrodes (ring diameter 8 mm, distance between rings 32 mm) and stimulated with square wave pulses of 1 ms duration from a Grass S 48 stimulator. Voltage was adjusted to at least 1.5 times the level which gave a maximum con-

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traction of the tissue. Frequency, unless otherwise indicated, was 6 Hz.

Cumulative concentration-response curves

Agonists were added to the bathing solution using logarithmic dosage increments (Van Rossum, 1963), allowing each dose to produce its full effect. A 30 min washout period was allowed between each curve, during which time the bathing fluid was changed at least four times. EC_{50} values were calculated and dose-ratios were estimated as the ratio of the EC_{50} after and before the modifying drug. pA_2 values were calculated as the intercept of the plot of the log (dose ratio − 1) against − log of the molar concentration of the modifying drug (Arunlakshana & Schild, 1959). The slope of the plot was measured by the method of least squares analysis.

Drugs

The following drugs were used: adenosine (Sigma); 2-chloroadenosine (Sigma); clonidine hydrochloride (Boehringer Ingelheim); cocaine hydrochloride; dopamine hydrochloride (Sigma); *cis*-flupenthixol (Lundbeck); haloperidol (Haldol; Janssen); (−)-isoprenaline hydrochloride (Sigma); metoclopramide hydrochloride monohydrate (Beecham); (−)-noradrenaline bitartrate (Boyer); phentolamine hydrochloride (CIBA-Geigy); (−)-phenylephrine hydrochloride (Sigma); procainamide hydrochloride (Pronestyl; Squibb): (±)-propranolol hydrochloride (Avlocardyl; ICI); sulpiride (Delagrange); sultopride hydrochloride (Delagrange); tetrodotoxin (Sankyo); tyramine hydrochloride (Sigma). Reserpine (Sigma) was dissolved as 1 mg/ml in 10% ascorbic acid.

Results

Clonidine and the vas deferens

Vas deferens preparations responded to field stimulation (6 Hz for 1 s at 15 s intervals) with a rapid, apparently monophasic, contraction. Developed tension declined by 20 to 50% over the first six contractions but remained stable thereafter. The responses were abolished by tetrodotoxin (0.3 μM, $n = 3$).

Cumulative addition of clonidine (1 to 30 nM) inhibited the tension developed in response to field stimulation in a reproducible, reversible and concentration-dependent manner. This effect appeared to be presynaptic, because even high concentrations of clonidine (370 nM, $n = 4$) did not antagonize submaximal contractions of the vas induced by phenylephrine (10 μM). Clonidine did not apparently inhibit the responses to field stimulation by an action on dopamine receptors, because neither haloperidol (0.1 μM, $n = 3$) nor *cis*-flupenthixol (1 μM, $n = 3$) antagonized the inhibitory effects of this compound. Higher concentrations of haloperidol (1 μM, $n = 3$) inhibited the responses to field stimulation directly and antagonized the contractile responses to phenylephrine (10 μM). Cimetidine (10 μM), a histamine H_2-receptor antagonist, did not antagonize the effects of clonidine.

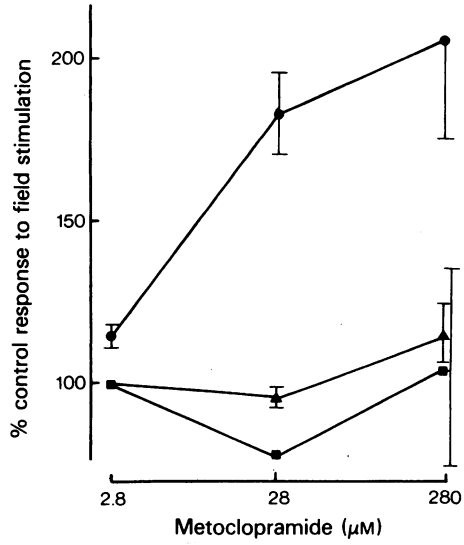

Figure 1 Augmentation of the response of the rat vas deferens to field stimulation (6 Hz for 1 s at 15 s intervals) by metoclopramide (●, n = 6–19). The effects of metoclopramide in tissues treated with phentolamine (0.1 μM, ▲, $n = 4$–9) and in tissues taken from rats pretreated with reserpine (2 mg/kg daily i.p. for 2 days, ■, $n = 5$) are also shown. Vertical bars represent s.e. mean. The developed tension (100%) was 711 ± 85 mg in the preparations treated with metoclopramide alone, 662 ± 70 mg in the preparations incubated with phentolamine and 480 ± 143 mg in the preparations taken from reserpine-treated animals.

The clonidine-induced inhibition of the response to field stimulation was blocked by phentolamine (pA_2 7.7 ± 0.2, slope −0.91 ± 0.10, $n = 5$). Phentolamine also antagonized the contractile effects of phenylephrine (pA_2 7.6 ± 0.1, slope −1.19 ± 0.18, $n = 4$), indicating that both types of responses were mediated by α-adrenoceptors. Phentolamine (0.1 to 1 μM) has minimal (< 10%) and inconsistent direct effects on the contractile responses to field stimulation in these experiments.

Effects of metoclopramide

Metoclopramide (2.8 to 280 μM) caused a concentration-dependent augmentation of the contraction induced by field stimulation (Figure 1). The

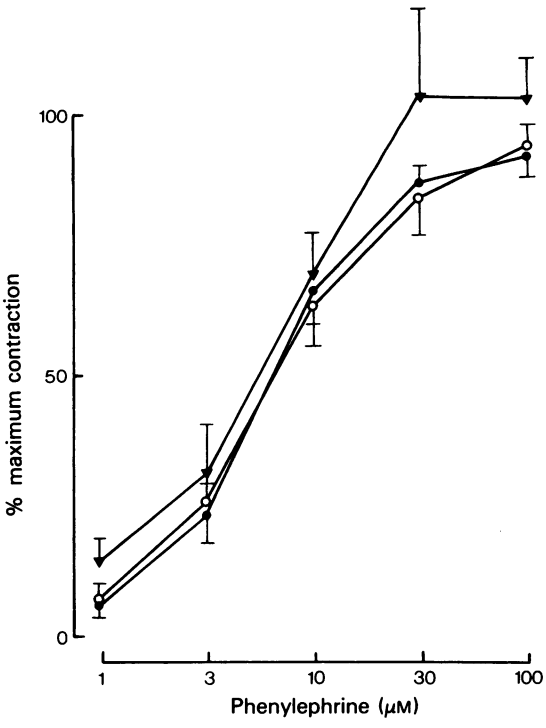

Figure 2 Effects of metoclopramide (initial controls ●; 28 μM ○; 280 μM ▼; 10 min incubation) on the contractile resonses of the rat vas deferens to phenylephrine. Phenylephrine was added cumulatively at 30 min intervals; maximum developed tension (100%) was 670 ± 53 mg. Vertical bars represent s.e. mean, $n = 4$. Note that metoclopramide did not affect the sensitivity of the preparations to phenylephrine.

effect was maximal within 3 min and was antagonized by phentolamine (0.1 μM; Figure 1). Metoclopramide caused little augmentation of the response to field stimulation in preparations from rats previously pretreated with reserpine (2 mg/kg/daily for 2 days, i.p.) and in some experiments caused a slight depression of the response (Figure 1). Metoclopramide (2.8 to 280 μM) did not produce a contractile effect by itself or affect the responsiveness of the rat vas deferens to phenylephrine (Figure 2), indicating that the drug had little effect on postsynaptic α-adrenoceptors.

The inhibitory effects of clonidine on the responses to field stimulation were antagonized by metoclopramide (2.8 to 280 μM). Cumulative concentration-response curves to clonidine were progressively displaced to the right in parallel by increasing concentrations of metoclopramide (Figure 3). The antagonism appeared to be competitive, the slope of the plot (log (dose ratio − 1) against − log molar concentration of antagonist) being −0.93 ± 0.08. The intercept yielded a pA_2 value of 5.3 ± 0.1. Similar pA_2 values were obtained if cocaine, 10 μM (pA_2 5.4 ± 0.1, slope −0.86 ± 0.06, $n = 4$), or hexamethonium, 100 μM (pA_2 5.5 ± 0.1, slope −0.99 ± 0.03, $n = 4$) were included in the Tyrode solution.

Specificity of the antagonism

Adenosine (1 to 100 μM) and 2-chloroadenosine (0.1 to 3 μM) inhibited responses to field stimulation but not phenylephrine-induced contractions. The inhibitory effects of these drugs were not antagonized by metoclopramide (2.8 to 280 μM, Figure 3).

Tyramine (60 μM) exerted two effects on the vas deferens. An initial inhibition of the responses to field

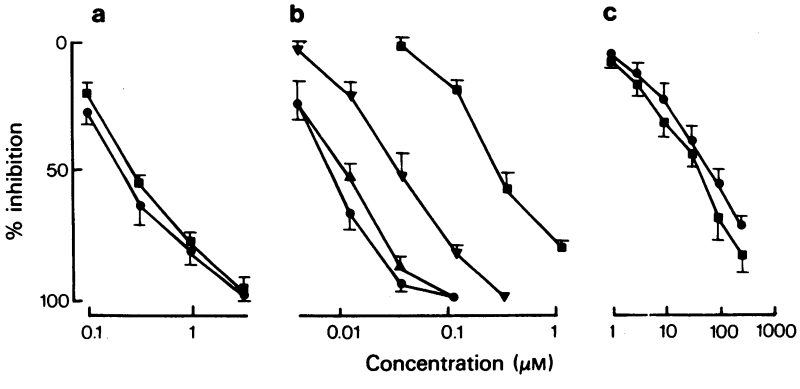

Figure 3 Effects of metoclopramide (2.8 μM ▲; 28 μM ▼; 280 μM ■) on the inhibitory effect of 2-chloroadenosine (a), clonidine (b), and adenosine (c), on the responses of the rat vas deferens to field stimulation (6 Hz for 1 s at 15 s intervals). The agonists were added cumulatively and concentration-response curves repeated at 30 min intervals. Control responses are indicated by (●). The effects of 2.8 μM and 28 μM metoclopramide on responses to 2-chloroadenosine and to adenosine are not shown; the values did not differ significantly from control values. Vertical bars represent s.e. mean, $n = 4$–6.

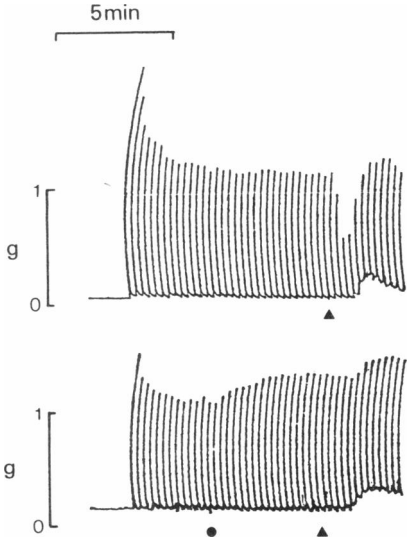

Figure 4 The effects of tyramine (▲, 60 μM) on contractile responses of contralateral rat vas deferens induced by field stimulation (6 Hz for 1 s at 15 s intervals). Metoclopramide (● 84 μM) was added 5 min before tyramine in the lower panel. Note that metoclopramide antagonized the inhibitory effects of tyramine, but not the contractile effects.

stimulation was followed by a contraction of the tissue. Metoclopramide antagonized the inhibitory effects of tyramine (Figure 4) but did not antagonize the tyramine-induced contraction, which is dependent upon uptake of tyramine into the adrenergic nerves (Greenberg & Long, 1971).

Effects of isoprenaline

(a) *Effects on β-adrenoceptors* (−)-Isoprenaline (0.047 to 1.42 μM) inhibited the submaximal contractions of the vas deferens induced by phenylephrine (25 μM) and the contractions induced by field stimulation (Figure 5). (±)-Propranolol (3.3 μM, Figure 5) antagonized these effects indicating that they were mediated by β-adrenoceptors. Neither phentolamine (0.1 μM, Figure 6a) nor metoclopramide (28 μM, Figure 6c) antagonized the propranolol-susceptible effects of isoprenaline on the responses to field stimulation.

(b) *Effects in the presence of β-adrenoceptor blockade* In the presence of (±)-propranolol (3.3. μM) high concentrations of isoprenaline (0.47 to 14 μM) did not inhibit responses to phenylephrine (Figure 5a) but did inhibit the responses to field stimulation. This inhibition was not affected by increasing the concentration of propranolol three fold (Figure 5b) and cannot be attributed to activation of postsynaptic β-adrenoceptors. Furthermore, (−)-isoprenaline (1 μM) inhibited (60 ± 7% inhibition, $n = 4$) the effect of short trains of low frequency pulses (6 Hz for 1 s) more than that of long trains of high frequency pulses (16 Hz for 5 s, 12 ± 7% inhibition, $n = 4$; 30 Hz for 5 s, <5% inhibition, $n = 2$).

Phentolamine (0.1 to 10 μM, Figure 6b, apparent pA_2 7.7 ± 0.3, slope −0.79 ± 0.08) and metoclopramide (2.8 to 280 μM, Figure 6d, pA_2 5.3 ± 0.2, slope −1.18 ± 0.12) antagonized the propranolol-resistant effects of isoprenaline.

Very high concentrations of (−)-isoprenaline (140 μM threshold) contracted the vas deferens directly. Complete concentration-response curves were not

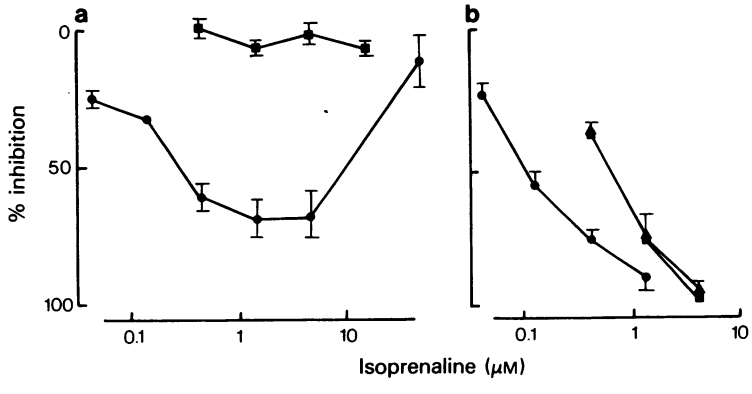

Figure 5 The inhibitory effects of (−)-isoprenaline (control ●) on contractile responses of the rat vas deferens induced by phenylephrine (25 μM) in (a) and by field stimulation (6 Hz for 1 s at 15 s intervals) in (b) and antagonism of these effects by (±)-propranolol (3.3 μM ■; 10 μM ▲). Vertical bars represent s.e. mean, $n = 4$.

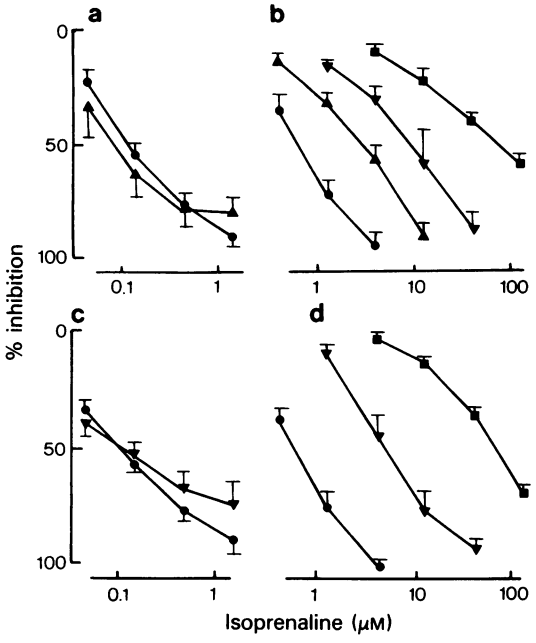

Figure 6 The inhibitory effects of isoprenaline (control ●) on contractile responses of the rat vas deferens induced by field stimulation (6 Hz for 1 s at 15 s intervals) in the presence of phentolamine (a and b, ▲ 0.1 μM; ▼ 1 μM; ■ 10 μM) or metoclopramide (c and d, ▼ 28 μM; ■ 280 μM). (±)-Propranolol (3.3 μM) was present in (b) and (d). Note that phentolamine and metoclopramide antagonized only the propranolol-resistant effects of isoprenaline. Vertical bars represent s.e. mean, $n = 4$.

obtained because of the low sensitivity of the preparations to the agonist. The contractions induced by (−)-isoprenaline (1.4 mM) were abolished by phentolamine (1 μM, $n = 4$).

Other substituted benzamides

Sultopride (pA_2 6.1 ± 0.1, slope −0.85 ± 0.09, $n = 5$) and sulpiride (pA_2 5.1 ± 0.2, slope −0.96 ± 0.08, $n = 4$) antagonized the inhibitory effects of clonidine on responses to field stimulation. Sultopride and sulpiride augmented the responses to field stimulation at the same concentrations that antagonized the effects of clonidine, i.e. sultopride was approx. 10 fold more potent than sulpiride. Procainamide (27 to 270 μM) neither antagonized the effects of clonidine nor augmented the effects of field stimulation in four experiments.

Discussion

Although the nature of the neurotransmitter(s) in the vas deferens is unresolved (Ambache & Zar, 1971; Swedin, 1971; Jenkins, Marshall & Nasmyth, 1977; McGrath, 1978), it is believed that the inhibitory effects of clonidine on the responses of rat vas deferens to field stimulation result from inhibition of neurotransmitter release. Thus, the degree of inhibition is dependent upon pulse frequency and upon the Ca^{2+} concentration of the medium (Drew, 1978) and clonidine inhibits noradrenaline release from the mouse vas deferens following low frequency stimulation (Marshall, Nasmyth & Shepperson, 1977). Furthermore, both epididymal and prostatic portions of the vas deferens are inhibited by clonidine (Brown, McGrath & Summers, 1979). In the present experiments the effects of clonidine were antagonized competitively by phentolamine, the pA_2 value being 7.7, which is similar to the values found previously for this tissue (Doxey *et al.*, 1977; Drew, 1977; Rhodes & Waterfall, 1978). These findings, and the lack of antagonism by haloperidol and *cis*-flupenthixol, agents that antagonize the effects of dopamine at other sites (Seeman, Chau- Wong, Tedesco & Wong, 1975; Burt, Creese & Snyder, 1976; Fuder & Muscholl, 1978; Starke, Reiman, Zumstein & Hertting, 1978), suggest that clonidine acts on presynaptic α-adrenoceptors and not dopamine receptors in the rat vas deferens (Vizi, Somogyi, Hadhazy & Knoll, 1973).

Metoclopramide antagonized competitively the inhibitory effects of clonidine on the responses to field stimulation, yet did not antagonize the inhibitory effects of adenosine. Adenosine (1 to 100 μM) inhibits electrically-evoked tritium release from a number of tissues which have been preincubated with [^{3}H]-noradrenaline (Hedqvist & Fredholm, 1976; Verhaeghe, Vanhoutte & Shepherd, 1977; Hedqvist & Fredholm, 1979; Mueller, Mosimann & Weiner, 1979) and inhibits neurotransmitter release in the rat vas deferens by a presynaptic mechanism which is not mediated via α-adrenoceptors (Clanachan, Johns & Paton, 1977). The effects of 2-chloroadenosine, which are not potentiated by inhibitors of adenosine deaminase or nucleoside uptake (Muller & Paton, 1979), were also resistant to metoclopramide; thus, the lack of effective antagonism was not due to its being masked by a concomitant enhancement which may have arisen from inhibition of metabolism. Furthermore, metoclopramide affected neither the contractile responses to phenylephrine nor the inhibitory postsynaptic effects of isoprenaline. The drug appears to be a specific presynaptic α-adrenoceptor antagonist in this preparation.

The effects of isoprenaline in the rat vas deferens were complex. Low concentrations of isoprenaline inhibited contractile responses to field stimulation and to phenylephrine by an action on β-adrenoceptors. This agrees with previous findings using this preparation (Ganguly & Bhattacharya, 1969; Vohra,

1979). The concentration-response curve of the inhibitory effects of isoprenaline on phenylephrine-induced contractions was parabolic. A similar relationship exists in the guinea-pig vas deferens. Here, the ineffectiveness of isoprenaline, at high concentrations, is due to its weak agonist effects on excitatory postsynaptic α-adrenoceptors which counter the inhibitory effects of β-adrenoceptor stimulation (Large, 1965; Spedding & Weetman, 1972). In the present experiments, the ability of high concentrations of isoprenaline to cause a phentolamine-susceptible contraction indicates that isoprenaline also activates postsynaptic α-adrenoceptors in the rat vas deferens.

In the presence of propranolol, isoprenaline inhibited responses to field stimulation at a 10 fold higher concentration than that required to inhibit before β-adrenoceptor blockade. This effect was presumably presynaptic because phenylephrine-induced contractions were not inhibited by isoprenaline in the presence of propranolol. These propranolol-resistant effects of isoprenaline were antagonized by metoclopramide and phentolamine and the pA_2 values obtained (5.3 and 7.7) were identical to those obtained when clonidine was used as the agonist, indicating that isoprenaline (>0.5 μM) may activate presynaptic α-adrenoceptors. Interestingly, isoprenaline (0.5 to 1 μM) hyperpolarizes rat sympathetic ganglia preparations by an action which is antagonized by phentolamine but not by propranolol (Brown & Caulfield, 1979), an effect on 'α_2'-adrenoceptors (Berthelson & Pettinger, 1977; Langer, 1977). In the vas deferens, since high (>140 μM) concentrations of isoprenaline are required to activate the α-adrenoceptors located on the smooth muscle cells, there is a measure of selectivity for the presynaptic site.

The same concentrations of metoclopramide, sultopride and sulpiride that antagonized the effects of clonidine, augmented the responses to field stimulation. It is unlikely that this was due to a non-specific effect on the smooth muscle cells because of the lack of effect of metoclopramide on phenylephrine-induced contractions or on the postsynaptic inhibitory effects of isoprenaline. The augmentation did not occur in the presence of phentolamine and was reduced, or absent, in reserpine-treated animals. The simplest explanation for these findings is that endogenous noradrenaline, released by field stimulation, activates the same inhibitory presynaptic receptors as does clonidine, reducing neurotransmitter release (Vizi *et al.*, 1973; Jenkins *et al.*, 1977). Metoclopramide, by acting as an antagonist at these receptors, would prevent the inhibition, thereby augmenting the contraction. The rapid decline in developed tension following repeated field stimulation (Figure 4) indicates that an endogenous inhibitory mechanism was activated during these experiments. Marshall, Nasmyth & Shepperson (1978) have shown, in the mouse vas deferens, that the inhibitory effects of tyramine on responses to field stimulation are mediated by displaced noradrenaline acting on presynaptic α-adrenoceptors. Thus, the ability of metoclopramide to antagonize the inhibitory effects of tyramine is evidence that metoclopramide may antagonize endogenously-released noradrenaline.

One argument against the hypothesis that metoclopramide augments the response to field stimulation by this mechanism is that phentolamine had only small and inconsistent effects on the response to field stimulation (see Drew, 1977). However, this drug has approximately the same potency as an antagonist of both pre- and postsynaptic receptors whereas metoclopramide did not antagonize postsynaptic α-adrenoceptors at any concentration tested.

Metoclopramide has been shown to increase nerve stimulation-induced noradrenaline release from rabbit ear artery preparations (Hope *et al.*, 1978). However, concentrations of 10 μM were required, a concentration which would block presynaptic α-adrenoceptors. Hope *et al.* (1978) demonstrated that low concentrations of metoclopramide (0.2 μM) antagonized the inhibitory effects of dopamine, but not of noradrenaline, on responses to nerve stimulation. In this context, Fuder & Muscholl (1978), using a perfused rabbit heart preparation, have concluded that although presynaptic dopamine receptors may be demonstrated by use of suitable agonists and antagonists, these receptors do not normally regulate noradrenaline release following sympathetic nerve stimulation.

The ability of metoclopramide to antagonize presynaptic α-adrenoceptors as well as dopamine receptors, indicates caution in the interpretation of the effects of this drug solely in terms of dopamine receptor blockade. However, the order of potency of the 2-substituted benzamides as antagonists of clonidine in the vas deferens (sultopride > metoclopramide > sulpiride) is different from their abilities to antagonize dopamine-induced vasodilatation in the dog kidney (sulpiride ≫ metoclopramide; Kohli *et al.*, 1978) and to induce prolactin release (sulpiride > metoclopramide; Meltzer, Simonovic, Fang, Piyakalamala & Young, 1978). Thus these compounds act at sites that are distinct from the presynaptic α-adrenoceptor in two classic tests for dopamine receptor function. On the other hand, the fact that the drugs are effective antagonists at each site indicates that some forms of dopamine receptor are structurally related to α-adrenoceptors.

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