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Pharmacological study of several effects of hydralazine in the bisected rat vas deferens

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We have studied several effects of hydralazine in the bisected rat vas deferens. Hydralazine produced a shift to the left of the concentration-response curve for noradrenaline, with potentiation of the maximal response in both portions of the vas deferens. In contrast it caused a shift to the right of the concentration-response curve for noradrenaline in preparations pretreated with cocaine (inhibitor of catecholamine neuronal uptake), and of the curve for methoxamine and for CaCl_2 (in depolarizing medium with K^+ 55 mM), in all cases with depression of the maximal response. Hydralazine enhanced the contractions induced by noradrenaline in Ca^{2+} -free medium, except in the presence of cocaine. It had no effect on [^3H]noradrenaline neuronal uptake into noradrenergic neurons of the vas deferens, nor did it affect basal or K^+ -induced $^{45}\text{Ca}^{2+}$ uptake. These results suggest that hydralazine potentiates the contractions elicited by noradrenaline by a mechanism other than blockade of the neuronal uptake of this catecholamine. Our results also suggest that the inhibition by hydralazine of the contractions elicited by Ca^{2+} (in Ca^{2+} -free depolarizing high- K^+ 55 mM solution) and by methoxamine is not due to an action on voltage-dependent Ca^{2+} channels, but may reflect an intracellular site of action.

Hydralazine; Vas deferens (bisected, rat); [^3H]Noradrenaline uptake; $^{45}\text{Ca}^{2+}$ influx

1. Introduction

Hydralazine is a direct vasodilator that, at supra-therapeutic dosages, antagonizes the action of a variety of vascular smooth muscle spasmogens on isolated preparations (Khayyal et al., 1981; Lipe and Moulds, 1981; Brown et al., 1983; Orallo et al., 1991) through an unknown mechanism (Eleno et al., 1987). However, systematic studies of the effects of hydralazine in non-vascular smooth muscle have not yet been carried out.

Previous studies have demonstrated that hydralazine inhibits the contractions induced by histamine in guinea pig intestine, by BaCl_2 in rabbit intestine and by adrenaline in guinea pig seminal vesicle (Druey and Tripod, 1967; Gross, 1977). It has also been reported that the relaxant response to hydralazine in vascular and non-vascular smooth muscle is inversely proportional to the density of sympathetic nerve terminals (Worcel, 1978; Brown et al., 1983). More recently, the

study of the effects of hydralazine on blood flow to tumours has been the subject of considerable interest (Hasegawa and Song, 1991).

In addition, it has been shown that the epididymal and prostatic fractions of the rat vas deferens differ anatomically (Anton et al., 1977), have different adrenergic innervations (Zieher and Jaim-Etcheverry, 1971; Celuch and Sloley, 1988) and, in part as a result of the latter, show different sensitivities to the effects of various drugs (Pennefather et al., 1974; Kasuya and Suzuki, 1979; MacDonald and McGrath, 1980; Sallés and Badía, 1991).

In view of these reports, and with the aim of further elucidating hydralazine activity in non-vascular smooth muscle, we have studied the effects of this drug (at the dosages customarily used by other authors) on neuronal uptake of [^3H]noradrenaline, on tension responses to CaCl_2 (in Ca^{2+} -free high- K^+ 55 mM solution), to noradrenaline and to methoxamine, and on $^{45}\text{Ca}^{2+}$ influx (basal and noradrenaline- or K^+ -induced) in the two halves of the rat vas deferens.

A preliminary account of this study has already been published in abstract form (Campos et al., 1991).

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2. Materials and methods

2.1. Tissue preparation

Male Sprague-Dawley rats (300–350 g) were killed by a blow on the head and exsanguinated. Whole vasa deferentia were removed, placed in a Petri dish with Krebs bicarbonate solution (KBS) of composition (in mM): NaCl, 119; KCl, 5; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 2.5; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.2; KH_2PO_4 , 1.2; NaHCO_3 , 25; ethylenediaminetetraacetic acid (EDTA) $\cdot 2\text{H}_2\text{O}$, 0.03; ascorbic acid 0.56; glucose, 11. The solution was maintained at 37°C and bubbled with carbogen (95% O_2 and 5% CO_2). After removal of connective tissue and blood vessels, the vasa deferentia were divided into prostatic and epididymal portions.

2.2. Contraction studies

2.2.1. General procedure

Vas deferens portions were set up in isolated organ baths containing 10 ml of KBS maintained at 37°C and bubbled with carbogen. The preparations were equilibrated at a resting tension of 0.5 g for at least 1 h, during which the physiological solution was replaced every 10 min. Organ responses were measured using a Letica TRI 110 isometric transducer and recorded on a Letica Unigraph 1000–506 polygraph.

2.2.2. Noradrenaline and methoxamine concentration-response curves

Cumulative concentration-response curves were obtained by the method of Van Rossum (1963). In experiments with noradrenaline, two reproducible concentration-response curves were obtained with only a 60-min delay to allow washout and to minimize the possibility of receptor desensitization. Following the control curves, the tissues were incubated with hydralazine for 10 min after which a third concentration-response curve was obtained. To study the effects of cocaine, the drug was added to the KBS after two control curves had been obtained with noradrenaline: two reproducible concentration-response curves were then obtained and the tissues were incubated with hydralazine for 10 min after which a fifth concentration-response curve with noradrenaline was obtained. Other tissues were subjected to the same procedures simultaneously, but omitting hydralazine.

In the experiments with methoxamine, successive concentration-response curves obtained from a single preparation were not reproducible. Control, hydralazine- and cocaine-treated curves were therefore obtained in different preparations. The drugs were administered 10 min before making the curve.

2.2.3. Ca^{2+} concentration-response curves

After the equilibration period of 60 min in KBS, the tissues were incubated for 30 min in Ca^{2+} -free depolar-

izing KBS (containing 50 mM of KCl instead of the equivalent amount of NaCl, to maintain osmolarity). CaCl_2 was then added to the bath in stepwise fashion. On obtaining two reproducible control concentration-response curves, the effects of hydralazine or cocaine (added 10 min prior to initiation of a third curve) were determined.

2.2.4. Studies in Ca^{2+} -free medium

To evaluate contraction in a Ca^{2+} -free medium, prostatic and epididymal portions were equilibrated for 60 min in KBS and then washed for 5 min with a Ca^{2+} -free solution (the same KBS, but without CaCl_2 and containing 0.5 mM EGTA) before a noradrenaline contraction was elicited. To study the effects of hydralazine, the preparations were further washed in KBS for 60 min (to fill the Ca^{2+} stores depleted by the first contraction). There was a further 5-min pre-incubation in Ca^{2+} -free solution before a suitable concentration of hydralazine was added, followed later by noradrenaline. Another set of experiments was carried out with the addition of cocaine to all the physiological solutions at the outset. Other tissues were subjected to the same procedures simultaneously, but omitting hydralazine.

2.3. [^3H]noradrenaline uptake

After an initial 60-min equilibration period in KBS containing β -oestradiol (10 μM) to block extraneuronal uptake, maintained at 37°C and bubbled with carbogen, prostatic and epididymal portions were incubated in the same solution containing 0.3 $\mu\text{Ci/ml}$ of [^3H]noradrenaline for 60 min. To investigate the action of hydralazine and/or cocaine, they were added to the KBS 10 min before, and during, the incubation period with tritium. At the end of each experiment, the tissues were removed, blotted dry, weighed and digested in 1 ml H_2O_2 (110 volumes) at 115°C for 90 min. After cooling, 5 ml of Ready Safe HP Beckman was added and the radioactivity was measured in a liquid scintillation counter (Beckman LS 3801) following standard control procedures.

2.4. $^{45}\text{Ca}^{2+}$ influx

Prostatic and epididymal portions of vas deferens were equilibrated for at least 60 min in KBS (CaCl_2 , 1.5 mM) maintained at 37°C and bubbled with carbogen. The tissues were then incubated for 5 min in a $^{45}\text{Ca}^{2+}$ -containing medium (0.5 $\mu\text{Ci/ml}$) with or without noradrenaline or K^+ (55 mM), to assess the effect of these vasoconstrictor agents on $^{45}\text{Ca}^{2+}$ uptake.

To evaluate the actions of hydralazine on basal and induced $^{45}\text{Ca}^{2+}$ uptake, the drug was added to the bath 10 min before and during the incubation period with

$^{45}\text{Ca}^{2+}$. Thereafter the preparations were washed for 20 min in 150 ml of an ice-cold La^{3+} solution (composition, in mM: NaCl , 118; KCl , 4.7; tris hydroxymethylaminomethane, 5; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.2; $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$, 50; glucose, 11), pH 6.8, in order to remove extracellular Ca^{2+} from the tissue. The tissues were then removed, blotted dry, weighed and digested in H_2O_2 , and their radioactivity was measured following the procedures described above.

2.5. Expression and statistical analysis of results

Unless otherwise specified, results shown in the text and figures are expressed as means \pm S.E.M. Statistical differences between two means ($P < 0.05$ or $P < 0.01$) were determined by Student's two-tailed t -test for paired or unpaired data. The contractile responses to vasoconstrictor agents (in the presence or absence of hydralazine) are expressed as absolute tensions (mg). pD_2 values (negative \log_{10} of the molar concentration of agonist required to elicit 50% of maximal response) of the vasoconstrictor agents and pD_2' values (negative \log_{10} of the molar concentration of antagonist required to cause a 50% depression of the maximal response to the agonist) were obtained using Van Rossum's (1963) method.

$[^3\text{H}]$ noradrenaline tissue uptake was calculated from the formula: $[^3\text{H}]$ noradrenaline uptake (nmol/kg wet tissue) = cpm in tissue/wet tissue weight (kg) \times nmol $[^3\text{H}]$ noradrenaline in 1 l solution/cpm in 1 l solution.

$^{45}\text{Ca}^{2+}$ tissue uptake was calculated from the formula: $^{45}\text{Ca}^{2+}$ uptake (nmol/kg wet tissue) = cpm in tissue/wet tissue weight (kg) \times nmol $^{45}\text{Ca}^{2+}$ in 1 l solution/cpm in 1 l solution. Note that the numerator of the second factor in this expression is the concentration of $^{45}\text{Ca}^{2+}$, not the total Ca^{2+} concentration.

2.6. Drugs, chemicals and radioisotopes

The following drugs were used: hydralazine hydrochloride (Ciba Geigy); (-)-noradrenaline bitartrate (Sigma); (\pm)-methoxamine hydrochloride (Gayoso-Wellcome); (-)-cocaine hydrochloride (Abelló); β -oestradiol (Sigma); EGTA (ethyleneglycol-bis-(β -aminoethyl ether) N,N,N',N' -tetraacetic acid; Sigma).

The radioisotopes used in this study were: $^{45}\text{Ca}^{2+}$ (New England Nuclear, specific activity 21.25 mCi/mg); 1-[7,8- ^3H]noradrenaline (Amersham International, specific activity 9.3 Ci/mmol).

Hydralazine and cocaine were dissolved in de-ionized water immediately prior to use. (-)-Noradrenaline bitartrate and (\pm)-methoxamine hydrochloride were prepared daily in de-ionized water from stock solutions (10 mM) kept at -20°C . Sodium bisulphite (0.2%) was added to the noradrenaline stock solution to prevent oxidation. β -Oestradiol was dis-

solved in 95% ethanol to make a stock solution of 10 mM, and aliquots of this solution were then diluted with de-ionized water prior to use. All chemicals used for the preparation of physiological solutions were of analytical grade.

3. Results

3.1. Influence of hydralazine or cocaine on basal conditions

Rat vas deferens (epididymal and prostatic portions) lacked spontaneous activity. Resting tone was not affected by hydralazine (10 μM to 1 mM) or cocaine (10 μM) ($P > 0.05$, $n = 6$).

3.2. Effects of hydralazine on contractions induced by noradrenaline

Noradrenaline elicited dose-related contractions in both the prostatic and epididymal portions of the vas deferens. The pD_2 values obtained were 5.84 ± 0.12 for the epididymal portion and 4.78 ± 0.14 for the prostatic portion, and the maximal tensions (mg) reached were 890.0 ± 28.0 and 355.7 ± 15.2 respectively ($P < 0.01$, $n = 6$). Hydralazine (10 μM to 0.1 mM) did not affect these values significantly ($P > 0.05$, $n = 6$). Hydralazine (1 mM) produced a shift to the left of the concentration-response curve for noradrenaline, with potentiation of the maximal response in both portions. The maximal tensions (mg) reached were 1076.2 ± 16.2 for the epididymal portion ($P < 0.01$, $n = 6$) and 584.9 ± 8.2 for the prostatic portion ($P < 0.05$, $n = 6$). Significant differences were observed between the effects of hydralazine on the two portions ($P < 0.01$, $n = 6$; see fig. 1a).

3.3. Effects of hydralazine on noradrenaline-induced contractions in the presence of cocaine

Cocaine (10 μM) shifted noradrenaline concentration-response curves to the left, with potentiation of maximal responses. The maximal tensions (mg) reached were 1148.0 ± 34.8 ($\text{pD}_2 = 7.15 \pm 0.08$) for the epididymal portion and 403.7 ± 18.0 ($\text{pD}_2 = 6.33 \pm 0.05$) for the prostatic portion ($P < 0.01$, $n = 6$). The percentage potentiation produced by cocaine was almost identical in both portions. In preparations pretreated with cocaine, hydralazine (1 mM) caused a shift to the right of the concentration-response curve for noradrenaline, with depression of the maximal response in the epididymal (556.3 ± 20.5 mg) and prostatic (158.9 ± 7.6 mg) portions ($P < 0.01$; $n = 6$). The pD_2' values were 2.99 ± 0.09 for the epididymal and 3.21 ± 0.08 for the prostatic portion (fig. 1 b).

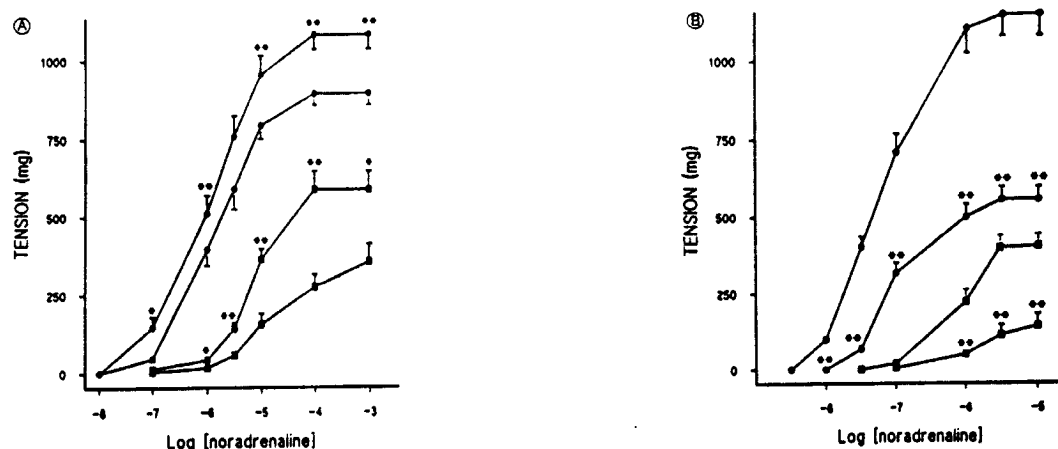


Fig. 1. (A) Cumulative concentration-response curves for noradrenaline in the epididymal (circles) and prostatic (squares) portions of the rat vas deferens in the absence (open symbols) or presence (solid symbols) of hydalazine (1 mM); (B) inhibitory effect of hydalazine (1 mM) (solid symbols) on the contractile responses to noradrenaline in the presence of cocaine (10 μ M) (open symbols) in the epididymal (circles) and prostatic (squares) portions of the bisected rat vas deferens. Level of statistical significance with respect to curves without hydalazine: * $P < 0.05$ or ** $P < 0.01$. Each point represents the mean value \pm S.E.M. (indicated by vertical lines) from at least six experiments.

3.4. Effects of hydalazine on methoxamine-induced contractions

Methoxamine produced concentration-dependent contraction in both epididymal and prostatic portions. The pD_2 values were 5.39 ± 0.13 for the epididymal and 4.74 ± 0.13 for the prostatic portion. The maximal tensions (mg) reached were 851.9 ± 26.0 and 389.4 ± 15.1 , respectively ($P < 0.01$, $n = 6$).

Hydalazine (1 mM) significantly inhibited methoxamine-induced contractions and caused a shift to the right of the concentration-response curves for this vasoconstrictor agent, with depression of the maximal response, in both portions. The maximal tensions reached were 487.6 ± 11.0 mg for the epididymal portion and 113.5 ± 8.5 mg for the prostatic portion ($P < 0.01$, $n = 6$). The pD_2' values were 2.87 ± 0.07 and 3.39 ± 0.04 , respectively. Significant differences were observed between the effects of hydalazine on the two portions ($P < 0.01$, $n = 6$; see fig. 2).

Cocaine (10 μ M) did not significantly modify the contractile response elicited by methoxamine either in the epididymal ($pD_2 = 5.46 \pm 0.09$) or the prostatic ($pD_2 = 4.60 \pm 0.15$) portions ($P > 0.05$ with respect to control values without cocaine, $n = 6$). The inhibitory effect of hydalazine was also unaffected by cocaine (10 μ M) ($pD_2' = 2.91 \pm 0.20$ and 3.25 ± 0.08 , respectively; $P > 0.05$ with respect to values without cocaine, $n = 6$).

3.5. Effects of hydalazine on $CaCl_2$ -induced contractions in K^+ -depolarized preparations

When both portions were exposed to Ca^{2+} -free high- K^+ (55 mM) solution, addition of Ca^{2+} to the bath produced a progressive increase in the tension

developed. The maximal tensions reached were 871.4 ± 22.9 in the epididymal and 489.9 ± 17.5 mg in the prostatic portion ($P < 0.01$, $n = 6$). The EC_{50} values (50% effective concentrations) were 1.50 ± 0.12 mM and 1.20 ± 0.12 mM, respectively.

Hydalazine (1 mM) produced a shift to the right of the $CaCl_2$ concentration-response curve with reduction of the maximal tension in both the epididymal (238.5 ± 54.4 mg) and the prostatic (247.8 ± 23.4 mg) portions ($P < 0.01$, $n = 6$). The pD_2' values were 3.49 ± 0.12 and 2.99 ± 0.07 , respectively. The differences between the effects of hydalazine on the two portions were significant ($P < 0.01$, $n = 6$; see fig. 3).

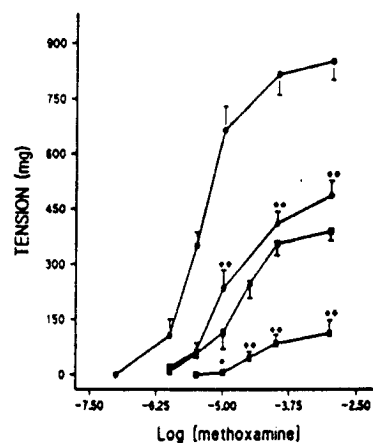


Fig. 2. Cumulative concentration-response curves for methoxamine in the epididymal (circles) and prostatic (squares) portions of the rat vas deferens in the absence (open symbols) or presence (solid symbols) of hydalazine (1 mM). Each point represents the mean value \pm S.E.M. (indicated by vertical lines) from at least six experiments. Level of statistical significance with respect to curves without hydalazine: * $P < 0.05$ or ** $P < 0.01$.

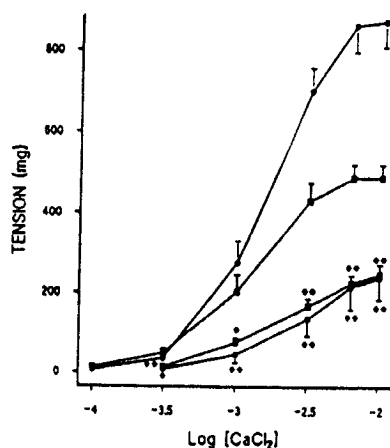


Fig. 3. Cumulative concentration-response curves for CaCl_2 in the epididymal (circles) and prostatic (squares) fractions of the rat vas deferens in the absence (open symbols) or presence (solid symbols) of hydralazine (1 mM). Each point represents the mean value \pm S.E.M. (indicated by vertical lines) from at least six experiments. Level of statistical significance: * $P < 0.05$ or ** $P < 0.01$ with respect to control curves.

Preincubation in cocaine (10 μM) did not significantly alter either the Ca^{2+} concentration-response curve or the inhibitory effect of hydralazine. ($\text{EC}_{50} = 1.53 \pm 0.02$ and $\text{pD}'_2 = 3.40 \pm 0.21$ for the epididymal portion; $\text{EC}_{50} = 1.18 \pm 0.10$ and $\text{pD}'_2 = 3.01 \pm 0.09$ for the prostatic portion; $P > 0.05$ with respect to curves with and without hydralazine in the absence of cocaine, $n = 6$).

3.6. Effects of hydralazine on noradrenaline-induced contractions in Ca^{2+} -free medium

In both portions, noradrenaline (1 mM) produced a characteristic contraction with two distinct components: an initial transient contraction (fast component) of 88.4 ± 3.1 mg in the epididymal portion and 101.6 ± 4.9 mg in the prostatic portion ($n = 6$), that relaxed to a sustained tension (slow component) of 33.3 ± 3.1 mg and 12.2 ± 1.6 mg, respectively ($P < 0.01$, $n = 6$). These contractions were enhanced by hydralazine (1 mM) in the epididymal portion (fast component tension = 112.7 ± 3.4 mg, $P < 0.01$, $n = 6$; slow component tension = 47.2 ± 2.9 mg, $P < 0.05$, $n = 6$) and in the prostatic portion (fast component tension = 162.0 ± 5.6 mg; slow component tension = 26.9 ± 1.9 mg; $P < 0.01$; $n = 6$).

In preparations pretreated with cocaine (10 μM), both fast and slow contractions were significantly enhanced in the epididymal portion (fast component tension = 114.8 ± 3.1 mg; slow component tension = 60.0 ± 4.1 mg; $P < 0.01$, $n = 6$) and in the prostatic portion (fast component tension = 128.0 ± 5.0 mg; slow component tension = 22.5 ± 1.6 mg, $P < 0.05$, $n = 6$). These contractions were inhibited by hydralazine (1 mM) in the epididymal portion (fast component tension = 56.3 ± 4.2 mg; slow component tension = 28.0 ± 4.3 mg; $P < 0.01$, $n = 6$) and in the prostatic portion (fast component tension = 59.8 ± 3.6 mg; slow component tension = 9.3 ± 1.3 mg; $P < 0.01$; $n = 6$; see fig. 4).

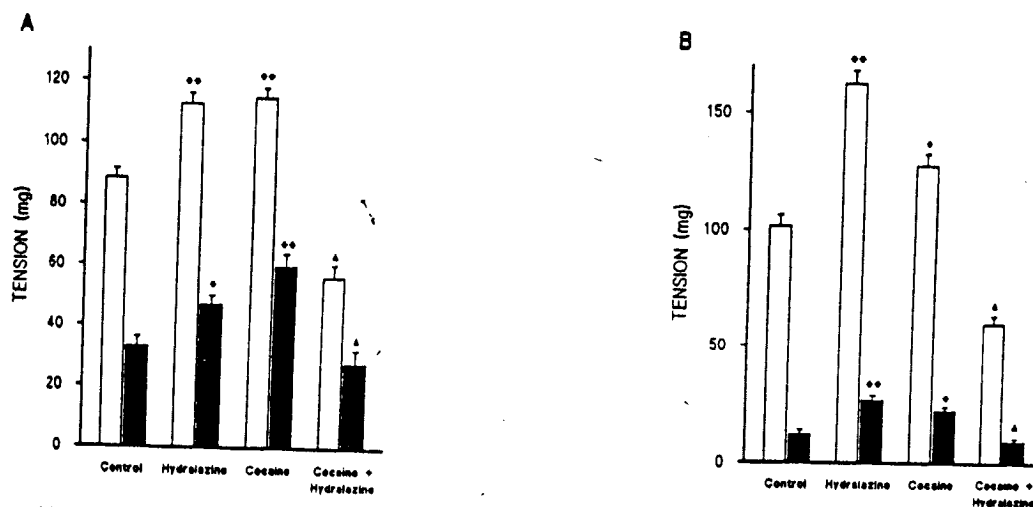


Fig. 4. Effects of hydralazine (1 mM) and cocaine (10 μM) on the fast (open bars) and the slow (solid bars) components of the contractions elicited by noradrenaline (1 mM) in the epididymal (A) and the prostatic (B) portion of the rat vas deferens in a Ca -free medium. Each column represents the mean value \pm S.E.M. (indicated by vertical lines) from at least six experiments. Level of statistical significance: * $P < 0.05$ or ** $P < 0.01$ with respect to controls and Δ $P < 0.01$ with respect to contractions in the presence of cocaine 10 μM .

TABLE 1

[³H]Noradrenaline uptake (nmol/kg) by the epididymal and prostatic portions of the rat vas deferens in the absence or presence of cocaine (10 μ M) and/or hydralazine (1 mM).

Each value is the mean \pm S.E.M. from at least six experiments.

	Epididymal	Prostatic
Control	327.6 \pm 25.4	226.2 \pm 23.5
Cocaine	149.7 \pm 8.0 *	106.3 \pm 13.5 *
Hydralazine	331.0 \pm 22.5	228.8 \pm 10.3
Cocaine + hydralazine	161.6 \pm 9.3 *	97.5 \pm 14.2 *

Statistical significance with respect to control: * $P < 0.01$.

3.7. Effects of hydralazine on [³H]noradrenaline neuronal uptake

[³H]Noradrenaline was actively taken up into sympathetic nerve terminals in both portions of the rat vas deferens. Cocaine (10 μ M) considerably inhibited noradrenaline uptake. This inhibitory effect was not modified in the presence of hydralazine (1 mM) ($P > 0.05$, $n = 6$). Furthermore, hydralazine (1 mM) displayed no significant inhibitory effect ($P > 0.05$, $n = 6$; table 1).

3.8. Effects of hydralazine on ⁴⁵Ca²⁺ uptake

Noradrenaline (1 mM) did not modify significantly ⁴⁵Ca²⁺ basal uptake by the epididymal and prostatic portions of the rat vas deferens ($P > 0.05$, $n = 6$), whereas high K⁺ (55 mM) increased the basal ⁴⁵Ca²⁺ uptake in both portions. The addition of hydralazine (1 mM) affected neither basal ⁴⁵Ca²⁺ influx ($P > 0.05$, $n = 6$) nor high K⁺-induced ⁴⁵Ca²⁺ uptake ($P > 0.05$, $n = 6$; table II).

4. Discussion

The results obtained in the present study show that, in the prostatic and epididymal portions of the rat vas deferens, unlike in vascular smooth muscle, hydrala-

zine (1 mM) increases the contractile response induced by noradrenaline in normal and Ca²⁺-free solution. However, when both portions were pre-incubated in cocaine (to block neuronal uptake of catecholamines), hydralazine inhibited noradrenaline-induced contractions in normal and Ca²⁺-free medium. Hydralazine did not modify the inhibition of [³H]noradrenaline uptake by cocaine, suggesting that it may potentiate noradrenaline-induced contractions by interfering with the neuronal uptake of catecholamines. Four observations support this hypothesis: (1) The relaxant action of hydralazine appears to be modulated by sympathetic nerve terminals present in vascular and non-vascular smooth muscle (Worcel, 1978; Brown et al., 1983). (2) In vascular smooth muscle (rat and rabbit aorta), in which hydralazine exhibits a strong inhibitory effect (McLean et al., 1978a,b; Khayyal et al., 1981), cocaine does not modify noradrenaline concentration-response curves (D'Agostino et al., 1983), contrary to our results in bisected rat vas deferens and to previous results with this tissue (Grana et al., 1980); such results are in accordance with the absence of a dense sympathetic innervation in rat thoracic aorta, reported by Patil et al. (1972), and with the presence of dense noradrenergic innervation in the rat vas deferens (Anton et al., 1977). (3) Hydralazine does not enhance but antagonizes the contractions induced by Ca²⁺ (in Ca²⁺-free high-K⁺ 55 mM solution) and methoxamine (which is not taken up into noradrenergic neurons). (4) The potentiation effect of hydralazine is greater in the prostatic portion than in the epididymal portion, which is less densely innervated (Zieher and Jaim-Etcheverry, 1971; Kasuya and Suzuki, 1979).

However, the results obtained show the lack of effects of hydralazine on neuronal uptake of [³H]noradrenaline into sympathetic nerve terminals in the prostatic and epididymal portions of the vas deferens. This indicates that the potentiation of noradrenaline-induced contraction produced by hydralazine in both halves of the rat vas deferens is not due to blocking of the neuronal uptake of catecholamines. It is possible that the potentiating effect is due to interference with a step in the metabolism of noradrenaline in noradrenergic neurons of the vas deferens; this interference may be inhibition of intramitochondrial MAO since, as suggested by Lyles et al. (1983), hydralazine inhibits rat liver mitochondrial MAO-A and MAO-B over the range 10⁻⁶ to 10⁻³ M. Such a hypothesis is supported by the fact that MAO activity is, according to Carvalho et al. (1991), greater in the prostatic portion.

In the present study, we found that, in each portion, hydralazine antagonizes in a non-competitive way and with almost equal effectiveness contractions induced by methoxamine, noradrenaline (in preparations pre-incubated in cocaine) and Ca²⁺ (in Ca²⁺-free high K⁺ 55 mM solution). This suggests that hydralazine acts intra-

TABLE 2

Basal, noradrenaline (1 mM)- and K⁺ (55 mM)-evoked ⁴⁵Ca²⁺ uptake (nmol/kg) by the epididymal and prostatic portions of the rat vas deferens. Effects of hydralazine (1 mM) in the absence of other agents or in the presence of K⁺ (55 mM).

Each value is the mean \pm S.E.M. from at least six experiments.

	Epididymal	Prostatic
Basal	66.7 \pm 4.1	45.2 \pm 6.1
Noradrenaline	60.0 \pm 7.2	46.5 \pm 5.0
K ⁺	113.4 \pm 6.0 *	87.3 \pm 3.7 *
Hydralazine	68.2 \pm 8.7	40.2 \pm 7.8
K ⁺ + hydralazine	109.6 \pm 3.5 *	81.5 \pm 4.2 *

Statistical significance with respect to basal uptake: * $P < 0.01$.

cellularly, as reported previously for human arteries and veins (Lipe and Moulds, 1981) and rat aorta (Orallo et al., 1991), possibly through a non-specific and receptor-independent interference with the excitation-contraction sequence at a point beyond receptor activation.

This hypothesis that hydralazine acts intracellularly is supported by the fact that hydralazine enters the cell in sufficient quantity to allow intracellular action (Baker et al., 1992) and by the results obtained in this work with Ca^{2+} -free medium in the presence of cocaine, in which hydralazine inhibited both fast noradrenaline-induced contractions – attributed to IP_3 -mediated release of Ca^{2+} from intracellular stores (Karaki and Weiss, 1988) – and the subsequent sustained contraction that is thought to involve the breakdown of phosphoinositide to diacylglycerol, the activation of protein kinase C by the latter and the induction of contraction in the presence of a low concentration of Ca^{2+} (Nishizuka, 1984). This intracellular action may be direct, via the following mechanisms: (1) Reduction of actin-myosin system activity (as previously reported by Jacobs, 1984 for bovine carotid arteries). (2) Inhibition of the release of intracellular Ca^{2+} . (3) Activation of Ca^{2+} uptake by intracellular stores. (4) Increase in the rate of Ca^{2+} loss (unlikely, given that hydralazine does not stimulate Ca^{2+} -dependent ATPase in rat aorta; Eleno et al., 1987).

It is unlikely that this intracellular action is due to an indirect effect via an increase in intracellular cGMP, since hydralazine does not increase cGMP levels in rat vas deferens (Diamond and Janis, 1978).

The fact that pD'_2 values for hydralazine in each portion were almost identical for contractions induced by methoxamine and by noradrenaline (in the presence of cocaine), and very close to the pD'_2 values for contractions induced by Ca^{2+} , would also tend to suggest the existence of a non-selective mechanism of hydralazine action on the cell membrane, involving the opening of K^+ channels and/or the blockage of Ca^{2+} influx through both voltage-dependent and receptor-operated Ca^{2+} channels. The effect of hydralazine on the smooth muscle cell membrane of rat vas deferens seems not to be due to its opening K^+ channels, as in previous studies in rat aorta (Orallo et al., 1991), since cromakalim and other K^+ channel openers do not antagonize contractions induced by K^+ at concentrations greater than about 30 mM (Clapham and Wilson, 1987; Cook and Quast, 1990).

Furthermore, the results obtained in the experiments involving $^{45}\text{Ca}^{2+}$ demonstrate that hydralazine has no effect on transmembrane voltage-dependent Ca^{2+} channels. In fact, basal and K^+ -induced $^{45}\text{Ca}^{2+}$ uptake is unaltered by the addition of hydralazine, in accordance with previous studies in rat aorta (Orallo et al., 1991), and in contrast with results obtained by

McLean et al. (1978b) and Weiss et al. (1981) in rabbit aorta. Our results suggest that hydralazine does not block transmembrane Ca^{2+} movements through either leak or voltage-dependent Ca^{2+} channels. This hypothesis is supported by a recent report that [^3H]hydralazine is not accumulated in the cell membrane (Baker et al., 1992).

On the other hand, the results of the present study shed no light upon the potential effects of hydralazine on receptor-operated Ca^{2+} channels, since noradrenaline did not significantly increase basal $^{45}\text{Ca}^{2+}$ uptake in either portion of the rat vas deferens, in accordance with the results of Khoyi et al. (1987, 1988) for guinea pig and rat vas deferens, and in contrast with the results of previous studies in rat aorta (Malta et al., 1986; Orallo et al., 1991; Gil-Longo et al., 1992).

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