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Effects of yohimbine, rauwolscine and corynanthine on contractions and calcium fluxes induced by depolarization and prostaglandin $F_{2\alpha}$ in rat aorta

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1 The effects of the selective α_2-adrenoceptor antagonists yohimbine and its stereo-isomer rauwolscine and the selective α_1-adrenoceptor antagonist corynanthine (a third yohimbine stereo-isomer) on contractions induced in rat aorta by depolarization and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) have been compared.

2 In calcium-free solution, depolarization with 100 mM K^+ failed to produce a contraction of rat aorta but $PGF_{2\alpha}$ (3 μM) stimulated a contraction equal to about 23% of maximal elicited in normal physiological solution.

3 Yohimbine had no significant effect on depolarization-induced contractions except at concentrations greater than 30 μM. Rauwolscine and corynanthine (1 to 100 μM) depressed depolarization-induced contractions in a concentration-dependent manner, but the characteristics of inhibition were not identical.

4 Contractions induced by $PGF_{2\alpha}$ (3 μM) were depressed in a concentration-dependent manner by rauwolscine (3 to 100 μM) but were unaffected by yohimbine or corynanthine.

5 Depolarization-stimulated ^{45}Ca influx was depressed by rauwolscine and corynanthine to about the same extent as were the contractions; while rauwolscine (100 μM) completely inhibited $PGF_{2\alpha}$-stimulated ^{45}Ca influx, it also depressed part of the $PGF_{2\alpha}$-stimulated contraction dependent on intracellular calcium.

6 Rauwolscine (100 μM) partly inhibited $PGF_{2\alpha}$-stimulated release of ^{45}Ca from aortic smooth muscle in calcium-free solution.

7 It is concluded that the yohimbine structure possesses a calcium entry blocking action as well as a depressant action on contractions not dependent on calcium entry. The predominant effect depends on the structural configuration and the nature of the stimulating agent.

Introduction

The α-adrenoceptor antagonist, yohimbine, has been described as relatively selective for prejunctional α_2-adrenoceptors rather than postjunctional α_1-adrenoceptors of smooth muscle (Starke, Borowski & Endo, 1975). A stereo-isomer of yohimbine, rauwolscine (or α-yohimbine) (Figure 1), is more selective for α_2-receptors than is yohimbine, while another stereo-isomer, corynanthine (or allo-yohimbine) (Figure 1), is a relatively selective antagonist of α_1-adrenoceptors (Starke, 1981; McGrath, 1982).

In the course of an investigation into the comparative responsiveness of the rat aorta to the stimulatory effects of a variety of α-adrenoceptor agonists and the relative dependence of these contractions on extracellular and intracellular calcium (Godfraind, Miller & Socrates Lima, 1982a), it became apparent that yohimbine seemed to antagonize noradrenaline-induced contractions in a competitive manner but that rauwolscine was not competitive (unpublished observations).

We have examined the effects of these three stereo-isomers on contractions induced in the rat aorta by K^+-induced depolarization and by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). These contractions are entirely (K^+-induced) or partly ($PGF_{2\alpha}$) dependent on extracellular calcium (Godfraind & Kaba, 1969; Godfraind & Miller, 1982), are not due to α-adrenoceptor

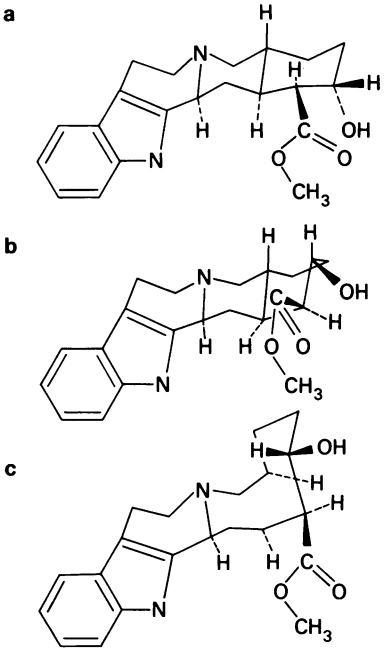

Figure 1 Representations of the steric configuration of: (a) yohimbine; (b) corynanthine; (c) rauwolscine.

stimulation and exhibit varying sensitivities to drugs blocking calcium entry (Godfraind & Dieu, 1981; Godfraind & Miller, 1983).

The results show that rauwolscine and corynanthine inhibit depolarization-induced contractions but not in an identical manner. Yohimbine had no significant effect on these contractions except at high concentrations. $PGF_{2\alpha}$-induced contractions were depressed by rauwolscine and unaffected by either yohimbine or corynanthine. The depressant effects of the compounds may be due in part to calcium entry blocking properties.

Some of these results have been communicated to the Société Belge de Physiologie et de Pharmacologie Fondamentales et Cliniques (Godfraind, Miller & Socrates Lima, 1982b).

Methods

Twelve to fifteen week-old female Wistar rats (weighing about 240 g) were killed by decapitation and the thoracic aorta removed and cleaned of all loosely adherent tissue. Rings of aorta about 2 mm wide were cut from close to the aortic arch (Godfraind, 1979) and suspended in 50 ml organ baths under a tension of 2 g. The physiological solution (composition: mM, NaCl 112, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 1.25, glucose 11.5) was maintained at 37°C and aerated with 95% O_2 and 5% CO_2. Calcium-free solution was prepared by omission of calcium. Contractile responses were measured with an isometric strain gauge coupled to a potentiometric pen recorder. After an equilibration period of 60 min, all preparations were contracted maximally either in a depolarizing medium (composition mM: NaCl 17, KCl 100, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 1.25, glucose 11.5) or by noradrenaline (1 μM), washed and allowed a further 60 min period for equilibration. Contractions induced by single concentrations of $PGF_{2\alpha}$ or by depolarization were then elicited at 90 min intervals.

In some experiments tissues were washed 3 times during a 10 min period with a calcium-free solution containing 0.2 mM EGTA before the second response to 3 μM $PGF_{2\alpha}$ was elicited.

To study the contractile effects of calcium, tissues were washed 3 times (in 10 min) with a calcium-free physiological solution before being washed with a calcium-free depolarizing solution. Cumulative concentration-effect curves to calcium (0.16 to 30 mM) were then obtained, after which preparations were washed in physiological solution until baseline tension was regained. After a further 30 min in this physiological solution the preparations were incubated with yohimbine, rauwolscine or corynanthine for 30 min before a second contraction was obtained as before. Control experiments in the absence of antagonists were performed at the same time.

Measurement of ^{45}Ca influx and efflux

The net rate of uptake of calcium into aortic muscle cells stimulated by depolarization or by $PGF_{2\alpha}$ (3 μM) was estimated by measuring the increase in ^{45}Ca content of the smooth muscle during exposure of the tissue to a physiological solution containing ^{45}Ca. Lanthanum, which has been shown to displace extracellular calcium while having little or no effect on intracellular calcium (van Breemen, Farinas, Gerba & McNaughton, 1972; Godfraind, 1974; 1976), was used to remove the relatively large amount of ^{45}Ca in the extracellular space. The latter would otherwise interfere with the determination of cellular ^{45}Ca content (see Godfraind, 1976).

The arteries were cut open longitudinally to form flat strips weighing about 6–11 mg and equilibrated for at least 60 min in physiological solution (composition mM: NaCl 122, KCl 5.9, $NaHCO_3$ 15, $MgCl_2$ 1.25, $CaCl_2$ 1.25, glucose 11) maintained at 37°C and aerated with a gas mixture of 95% O_2 and 5% CO_2. After preincubation for 30 min in physiological solution containing either yohimbine, rauwolscine or corynanthine (10, 30 or 100 μM), the artery strips were incubated for 5 min in 100 ml of physiological solution containing ^{45}Ca (1 μCi ml^{-1})

and either yohimbine, rauwolscine or corynanthine, then for another 2 min in a depolarizing solution (composition mM: NaCl 27, KCl 100, $NaHCO_3$ 15, $MgCl_2$ 1.25, $CaCl_2$ 1.25, glucose 11) also containing yohimbine, rauwolscine or corynanthine. A similar procedure was followed using $PGF_{2\alpha}$ (3 μM) to stimulate the influx of ^{45}Ca, except that a 3 min exposure to $PGF_{2\alpha}$ was used and a 4 min preincubation in ^{45}Ca physiological solution. Thereafter, preparations were washed for 5 min in 500 ml of a La^{3+} solution (composition mM: NaCl 122, KCl 5.9, $MgCl_2$ 1.25, $LaCl_3$ 50, glucose 11, Tris maleate 15 (pH 6.8)) to remove extracellular Ca^{2+} from the tissue. Parallel control experiments were always performed at the same time. After the La^{3+} wash, the artery strips were placed between two sheets of filter paper and pressed three times with a roller weighing 350 g. Each strip was weighed and dissolved in 0.1 ml of a solution composed of equal parts of perchloric acid (37% w/v) and H_2O_2 (30 vol) by heating for 15 min at 75°C. After cooling, 5 ml of Aqualuma (Lumac) was added and the radioactivity of the samples counted in a liquid scintilation counter. The results of each determination were converted to the apparent tissue content of Ca (mmol kg^{-1} wet wt) (Godfraind, 1976).

Efflux of ^{45}Ca from aortic strips stimulated by $PGF_{2\alpha}$ was estimated after they had been preincubated in ^{45}Ca (3 μCi ml^{-1}) containing physiological solution for 120 min, rauwolscine (100 μM) being present for the last 30 min. Tissues were rinsed for 4 min in a large volume of non-radioactive physiological solution containing rauwolscine before being transferred to a non-radioactive physiological solution containing rauwolscine and $PGF_{2\alpha}$ (3 μM) for 3 min. Tissues were thereafter treated as for ^{45}Ca influx experiments. Efflux experiments were also carried out at the same time in the absence of rauwolscine.

Drugs

Yohimbine HCl (Federa), rauwolscine HCl (Roth) and corynanthine HCl (Sigma) were dissolved in

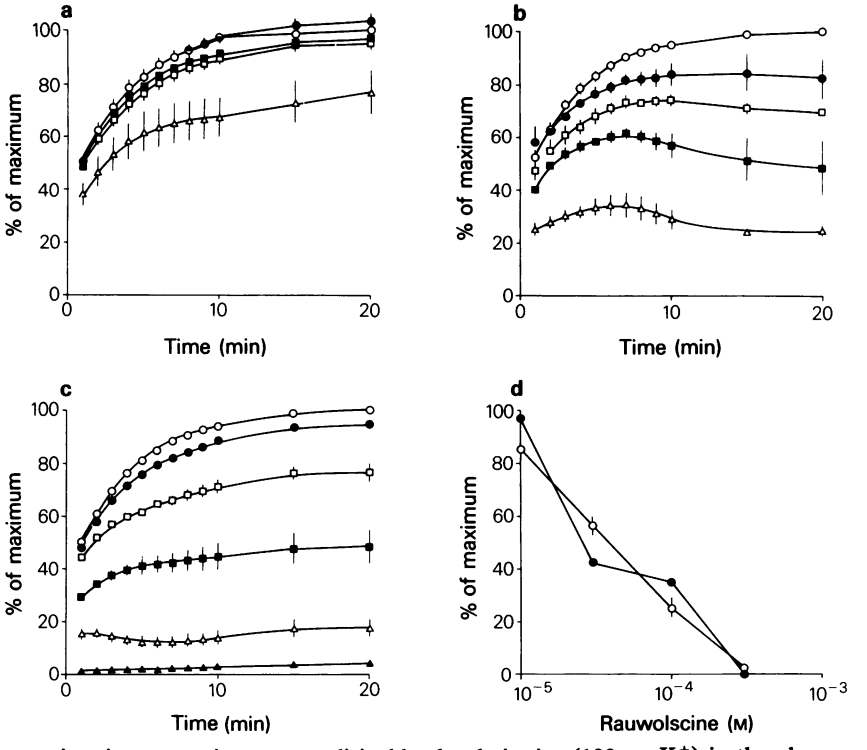

Figure 2 Contraction-time curves in rat aorta elicited by depolarization (100 mM K^+) in the absence (○) or presence of increasing concentrations [(●) 3 μM, (□) 10 μM, (■) 30 μM, (△) 100 μM, (▲) 300 μM] of yohimbine (a), corynanthine (b) and rauwolscine (c). Graph (d) represents the residual contraction (○) or influx of ^{45}Ca (●) stimulated by 2 min depolarization (100 mM K^+) in the rat aorta in the presence of various concentrations of rauwolscine. The ordinate scale in all cases represents the response in the presence of antagonist as a percentage of the maximal response attained in the absence of antagonist. Each curve is the mean of at least 4 observations. Vertical bars indicate s.e.mean when it exceeds symbol size.

distilled water to a concentration of 10 mM and further diluted as required. $PGF_{2\alpha}$ tromethamine salt (Dinolytic, Upjohn) was used as delivered. Noradrenaline bitartrate (Flucker) was dissolved in distilled water containing 7.9 mM Na_2SO_3 and 34 mM HCl as a stock solution of 10 mM and diluted in distilled water before use. ^{45}Ca (specific activity about 2 mCi μmol^{-1}) was obtained from the Radiochemical Centre, Amersham. All drug concentrations are expressed in terms of the base.

Statistical analysis

The data are expressed as means ± s.e.mean. In the case of experiments involving ^{45}Ca, the number of observations (n) applies to both control and non-control groups. Tests of significance have been made using Student's t test or a paired t test where possible; P values smaller than 0.05 were considered significant. The concentration of rauwolscine or corynanthine producing a 50% inhibition of the maximal contractile response (IC_{50}) or the maximal $PGF_{2\alpha}$ or depolarization-dependent ^{45}Ca influx (I_{50}) and their s.e. were estimated from concentration-effect curves.

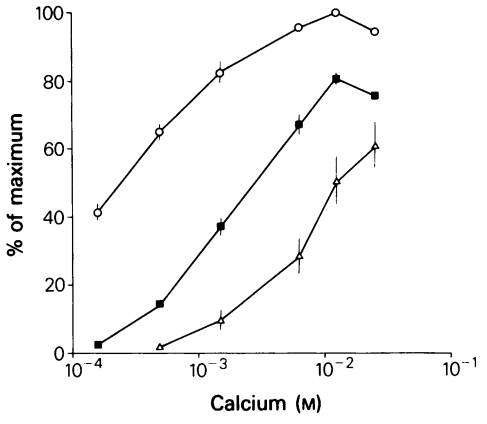

Figure 3 Concentration-effect curves elicited by calcium in depolarized (100 mM K^+) rat aorta in the absence (○) and presence of rauwolscine 30 μM (■) and 100 μM (△). Each point is the mean of at least 4 observations. Vertical bars indicate s.e.mean when it exceeds symbol size. Ordinate scale: percentage of maximal response obtained in the absence of rauwolscine.

Results

Depolarization-induced contractions

Contractions of rat aorta elicited by exposure to depolarizing solution were reproducible when separated by 60 min. These contractions were depressed by corynanthine (3 to 100 μM) in a concentration-dependent manner, the depressed contractions being characterized by an increase in the degree of inhibition with time of exposure to the depolarizing solution (use-dependent effect). A stable degree of inhibition was achieved after about 20 min (Figure 2b). The IC_{50} values for corynanthine measured 2 and 20 min after initiation of contractions were about 50 and 28 μM, respectively (Table 1).

Rauwolscine (3 to 100 μM) also depressed these contractions in a concentration-dependent manner. No use-dependent effect of rauwolscine was observed (Figure 2c) and the IC_{50} values of rauwolscine calculated 2 and 20 min after the initiation of contraction were about 33.9 and 27 μM, respectively (Figure 2d, Table 1) Yohimbine (0.3 to 30 μM) did not significantly affect these contractions but they were depressed by 23.1 ± 8.1% ($n = 3$, $0.01 < P < 0.05$) in the presence of yohimbine (100 μM) (Figure 2a).

Calcium-induced contractions

Concentration-effect curves produced by the cumulative addition of calcium (0.16 to 25 mM) to artery preparations washed in calcium-free depolarizing solution were reproducible when separated by 60 min. A maximal contraction was produced by about 12.5 mM calcium and was equal to 111.9 ± 5.0% ($n = 8$) of the contraction induced by noradrenaline (1 μM) (Figure 3). The calcium EC_{50} value was 0.31 ± 0.05 mM ($n = 8$). Concentrations of calcium greater than 12.5 mM reduced the maximal contraction.

In the presence of rauwolscine (30 and 100 μM), the calcium concentration-effect curves were depressed and maximal contractions were equal to

Table 1 Relative sensitivities to corynanthine, yohimbine and rauwolscine of contractions induced in the rat aorta by depolarization (100 mM K^+) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 3 μM) measured at equilibrium

	IC_{50} values		
	Corynanthine	*Yohimbine*	*Rauwolscine*
100 mM K^+	28 μM	Resistant	27 μM
$PGF_{2\alpha}$ (3 μM)	Resistant	Resistant	15 μM

80.6 ± 2.1% and 60.9 ± 6.7%, respectively, of that produced in the absence of rauwolscine (Figure 3).

Prostaglandin $F_{2\alpha}$-induced contractions

Contractions of rat aorta induced by $PGF_{2\alpha}$ (3 μM) were monophasic and reproducible when separated by 60 min, the induced contractions being equal to 83.1 ± 4.1% ($n = 24$) of the maximal contraction induced by noradrenaline (1 μM). These contractions were not significantly affected by the presence of yohimbine or corynanthine (0.3 to 100 μM). In the presence of rauwolscine (1 to 100 μM), contractions induced by $PGF_{2\alpha}$ were depressed in a concentration-dependent manner (Figure 4). No use-dependent effect was seen. The IC_{50} value for rauwolscine was calculated (at 20 min) to be about 15 μM (Table 1). In calcium-free solution, $PGF_{2\alpha}$ (3 μM) stimulated sustained monophasic contractions which amounted to 22.5 ± 3.8% ($n = 8$) of maximal contractions elicited in normal physiological solution. Because the residual contraction in the presence of rauwolscine amounted only to 8% of the control (Figure 4), it appears that rauwolscine depressed not only that part of the contraction dependent on extracellular calcium but also that part dependent on the release of intracellularly stored calcium.

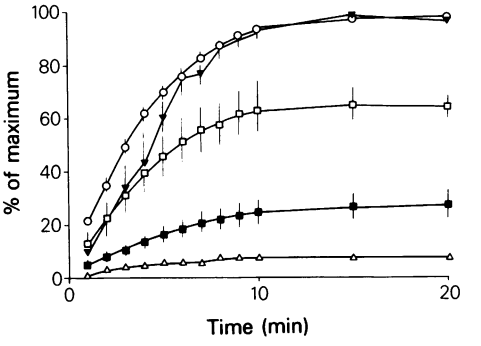

Figure 4 Contraction-time curves in rat aorta elicited by prostaglandin $F_{2\alpha}$ (3 μM) in the absence (○) and presence of 1 μM (▼), 10 μM (□), 30 μM (■) and 100 μM (△) rauwolscine. Each point is the mean of at least 3 observations Vertical bars represent s.e.mean when it exceeds symbol size.

^{45}Ca influx and efflux

Stimulation by depolarization Resting uptake of ^{45}Ca was not significantly affected by 100 μM yohimbine, corynanthine or rauwolscine. The uptake of ^{45}Ca stimulated by a 2 min exposure to depolarizing solution was about 118 μM calcium kg^{-1} wet wt. This stimulated influx was depressed in a concentration-dependent manner by rauwolscine (Table 2), the degree of depression correlating with the degree of inhibition of contraction measured at the same time interval (Figure 2d). Yohimbine (100 μM) depressed ^{45}Ca influx by about 11%, and corynanthine (30 μM) depressed the uptake by about 22%. These depressant effects on ^{45}Ca influx also correlate with the degree of depression of contraction measured at the same (2 min) time interval (Figure 2a, b).

Stimulation by prostaglandin$_{2\alpha}$ The uptake of ^{45}Ca stimulated by a 3 min exposure to $PGF_{2\alpha}$ (3 μM) was approx. 27 μM calcium kg^{-1} wet wt (tissue content after 4 min of passive exchange and 3 min stimulation by $PGF_{2\alpha}$ was 109.8 ± 4.1 μmol kg^{-1} wet wt) (Table 2). Corynanthine (100 μM) had no significant effect on this $PGF_{2\alpha}$-stimulated uptake of ^{45}Ca or on $PGF_{2\alpha}$-stimulated contractions measured after 3 min

Table 2 Mean tissue content of calcium (μmol kg^{-1} wet wt ± s.e.mean) in rat aorta measured as ^{45}Ca uptake into the lanthanum resistant calcium fraction

Rauwolscine (μM)	*Control*	*K^+-depolarization*
0	82.2 ± 1.6	200.0 ± 4.7
10	82.2 ± 3.2	196.3 ± 4.7
30	85.2 ± 5.6	135.0 ± 3.2
100	83.4 ± 7.1	132.9 ± 7.5
300	83.1 ± 1.6	79.9 ± 0.7
Rauwolscine		*$PGF_{2\alpha}$*
0	82.4 ± 4.7	109.8 ± 4.1
100	73.5 ± 1.2	73.3 ± 1.7
Corynanthine		*$PGF_{2\alpha}$*
0	80.3 ± 6.9 (6)	120.7 ± 3.0 (6)
100	84.8 ± 9.3 (4)	116.3 ± 6.0 (4)

Measured after 7 min of passive exchange (control) and after 5 min of passive exchange followed by 2 min of 100 mM K^+ stimulated uptake or by 3 min $PGF_{2\alpha}$ (3 μM). Unless otherwise stated, each value is the mean from at least 6 aortae.

Table 3 Mean tissue content of calcium (μmol kg^{-1} wet wt $\pm$ s.e.mean) in rat aorta measured as ^{45}Ca content of the lanthanum-resistant calcium fraction

Rauwolscine (μM)	*Control*		*$PGF_{2\alpha}$*
0	182.1 $\pm$ 4.3 (9)	$0.001 < P < 0.01$	151.8 $\pm$ 3.4 (10)
	$0.1 < P$ NS		$0.01 < P < 0.05$
100	191.8 $\pm$ 7.8 (12)	$0.01 < P < 0.05$	168.7 $\pm$ 5.2 (11)

Preincubation for 120 min was followed by either a 7 min wash (control) or by a 4 min wash and 3 min exposure to $PGF_{2\alpha}$ (3 μM)

contact. Rauwolscine (100 μM) depressed $PGF_{2\alpha}$-stimulated influx of ^{45}Ca.
Rauwolscine (100 μM) depressed $PGF_{2\alpha}$ (3 μM) stimulated efflux of ^{45}Ca from preloaded tissues from about 30 to 23 μmol kg^{-1} wet wt or by about 24% (Table 3).

Discussion

Receptor-response coupling can be altered not only by compounds interacting with membrane receptors, but also by actions on the chain of events between the binding of an agonist to its receptor and the activation of actomyosin-ATPase which follows.

Calcium entry blockers have been defined, as the name implies, as those compounds which inhibit the stimulated influx of calcium into smooth muscle cells and commonly have little or no effect on the unstimulated exchange of calcium across the membrane (Godfraind, 1981; Godfraind & Dieu, 1981; Godfraind & Miller, 1982; Godfraind, Miller & Socrates Lima, 1982a).

In vascular smooth muscle, agonist-induced contractions are, in some cases, accompanied by a depolarization (Bolton, 1979) and in other cases not (Somlyo & Somlyo, 1968; Keatinge, 1972; Casteels, 1980; Holman & Suprenant, 1980). However, in every muscle so far examined, physiological solutions containing more than 20 mM potassium depolarize the cell membrane and evoke a contraction that is totally dependent on extracellular calcium (Bolton, 1979).

Yohimbine has been shown here to exert no significant effect on depolarization-induced contractions except at high concentrations, while its two stereo-isomers rauwolscine and corynanthine exert, at equilibrium, a concentration-dependent depression of these contractions, their IC_{50} concentrations being similar when measured after 20 min (Table 1). However, the rate of onset of inhibition varies with the two isomers, corynanthine exhibiting a slight use-dependency (Godfraind & Dieu, 1981; Godfraind & Miller, 1982) while rauwolscine does not (Figure 2b and c). It has previously been argued that a use-dependent process, which is not observed with noradrenaline- and $PGF_{2\alpha}$-induced contractions but only with depolarization-induced contractions, indicates a change in the interaction of calcium entry blockers with cell membranes when they are depolarized (Godfraind & Dieu, 1981; Godfraind *et al.*, 1982a; Godfraind & Miller, 1983). The lack of a use-dependent effect with rauwolscine might simply indicate that its ability to bind to the membrane does not change with depolarization while that of corynanthine does.

Rauwolscine depressed Ca^{2+} concentration-effect curves and rauwolscine and corynanthine depressed depolarization-induced contractions, there being a good correlation between the degree of inhibition of calcium entry (Figure 2d).

Prostaglandin $F_{2\alpha}$-induced contractions of rat aorta are not thought to be due to an interaction with vascular α-adrenoceptors and cannot be secondary to liberation of noradrenaline, since the rat aorta is not sympathetically innervated (Patil, Fudge & Jacobowitz, 1972). When contractions were induced by $PGF_{2\alpha}$ in the presence of the yohimbine stereoisomers, a further separation of properties of the molecules was seen. Corynanthine and yohimbine had no significant effect, while rauwolscine (100 μM) abolished ^{45}Ca entry and depressed contractions by about 92%, having a similar potency as an inhibitor of contraction to that exhibited against depolarization-induced contraction (Table 1). However, if rauwolscine simply abolished calcium influx, then it could be expected that $PGF_{2\alpha}$-induced contractions would be depressed to about the same degree as by calcium-free solution, i.e. by about 77%. However, they were depressed by about 92% (Figure 3), indicating a further action of rauwolscine. This seems to be at least partially an interference with

the ability of $PGF_{2\alpha}$ to release intracellular calcium.

The complete lack of effect of corynanthine and yohimbine on contractions induced by $PGF_{2\alpha}$ and of yohimbine on depolarization-induced contractions indicates that the sites of interaction with the calcium gating mechanism activated by depolarization and by $PGF_{2\alpha}$ are stereo-specific.

The yohimbine stereo-isomers therefore seem to possess a calcium entry blocking action as well as a depressant effect on contractions unrelated to blockade of calcium entry. These effects depend on structural configuration and the nature of the stimulating agent. These actions have been observed at concentrations of rauwolscine close to those required for the antagonism of the postjunctional α-adrenoceptors (Starke, 1981). Calcium entry blockade additional to α-adrenoceptor blockade cannot therefore be ruled out when rauwolscine is used at micromolar or at higher concentrations.

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