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Pre- and Postsynaptic Effects of Yohimbine Stereoisomers on Noradrenergic Transmission in the Pulmonary Artery of the Rabbit

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Summary. Effects on noradrenergic neurotransmission of five stereoisomers of yohimbine and of the closely related compound yohimbol were studied in strips of the pulmonary artery of the rabbit. In some experiments the tissue was preincubated with ^{3}H-noradrenaline. Three effects were observed. Firstly, antagonism to the contractile effect of noradrenaline and of sympathetic nerve stimulation; the antagonism reflected competitive blockade of postsynaptic α-adrenoceptors. Secondly, an increase in the stimulation-evoked overflow of total tritium and ^{3}H-noradrenaline; the increase appeared to be due to blockade of presynaptic α-adrenoceptors. Thirdly, an increase in the basal outflow of ^{3}H-3,4-dihydrophenylglycol, presumably by impairment of the vesicular storage of ^{3}H-noradrenaline. According to their relative potencies in eliciting these effects, the drugs could be divided into three groups. Rauwolscine, β-yohimbine and yohimbol preferentially blocked the presynaptic α-adrenoceptor; rauwolscine and β-yohimbine, like yohimbine, at low concentrations increased the contractile response to sympathetic nerve stimulation. Corynanthine preferentially blocked the postsynaptic α-adrenoceptor. Pseudoyohimbine and 3-epi-α-yohimbine were very weak antagonists at either receptor; they mainly accelerated the basal outflow of ^{3}H-3,4-dihydroxyphenylglycol.

From these results and those of a previous study it is concluded that, in a series of twelve α-adrenolytic drugs, rauwolscine shows the greatest preference for presynaptic and corynanthine the greatest preference for postsynaptic α-adrenoceptors. In view of the chemical similarity of the two compounds these opposite properties are striking. Corynanthine and rauwolscine might be useful tools for the subclassification of α-adrenoceptors.

Key words: Yohimbine stereoisomers – Rauwolscine – Corynanthine – Presynaptic α-adrenoceptors – Postsynaptic α-adrenoceptors.

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Introduction

In many sympathetically innervated tissues, the presynaptic α-adrenoceptors which mediate inhibition of noradrenaline release, and the postsynaptic α-adrenoceptors which mediate the response of the effector cells, differ in their sensitivity to agonists (Starke, 1972; Starke et al., 1974, 1975b; Drew, 1977; Marshall et al., 1978; Doxey, 1979) and antagonists (Langer, 1973; Dubocovich and Langer, 1974; Starke et al., 1975a; Borowski et al., 1977; Drew, 1977; Blakeley and Summers, 1978; Butler and Jenkinson, 1978; Constantine et al., 1978; Kapur and Mottram, 1978; Marshall et al., 1978). In the pulmonary artery of the rabbit, yohimbine preferentially blocks presynaptic α-receptors; at low concentrations, it selectivity facilitates the release of noradrenaline and, in contrast to what one would expect from the classical postsynaptic antagonist effect, *enhances* contractions evoked by sympathetic nerve stimulation (Starke et al., 1975a). In a series of antagonists, yohimbine showed the greatest preference for the presynaptic receptors (Borowski et al., 1977).

In a search for further selective antagonists we studied some stereoisomers of yohimbine. The experiments were carried out on strips of the rabbit pulmonary artery. Some results have been reported to the German Pharmacological Society (Weitzell et al., 1979), and some findings with rauwolscine have been published in short form (Tanaka et al., 1978).

Methods

In the essentials, the methods corresponded to those used in our previous studies on the rabbit pulmonary artery (Starke et al., 1974; Borowski et al., 1977). The main pulmonary artery was spirally cut into a strip. The strip was incubated in or superfused with a medium containing (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 1.6, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, ascorbic acid 0.3, Na_2EDTA 0.03. The medium was equilibrated with 5% CO_2 in O_2 and warmed to 37°C. Cocaine 3×10^{-5} M, corticosterone 4×10^{-5} M and propranolol 4×10^{-6} M were added in some experiments specified under

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Results in order to block amine uptake mechanisms and β-adrenoceptors, respectively (Borowski et al., 1977). Isometric contractions were recorded on a Rikadenki pen recorder.

Isotope Experiments. Two artery strips were incubated for 1 h in 1.5 ml medium containing ^{3}H-noradrenaline 2.6×10^{-6} M, specific activity 2.2 – 2.8 Ci/mmole. Subsequently, each strip was mounted vertically between platinum wire electrodes under 2 p (= 19.6 mN) of tension and superfused with ^{3}H-noradrenaline-free medium for 210 min. The rate of superfusion was 2 ml/min. The superfusate was collected in 3-, 6- or 10-min samples. At the end, the strips were solubilized in 1 ml Soluene-100 (Packard Instrument). Tritium was measured by liquid scintillation spectrometry. Quenching was monitored by addition of known amounts of ^{3}H-toluene to representative samples.

For electrical stimulation of the sympathetic neurones, square wave pulses of 0.3 ms duration and 200 mA current strength were delivered to the tissue from a Stimulator II (Hugo Sachs Elektronik). Unless stated otherwise, the stimulation frequency was 2 Hz. Four stimulation periods of 3 min each were applied. They began after 132 (S_1), 153 (S_2), 174 (S_3) and 195 min (S_4) of superfusion. The stimulation-evoked overflow of total tritium was calculated by subtraction of the basal outflow and was expressed as per cent of the tritium content of the tissue at the onset of the respective stimulation period (Borowski et al., 1977). Solutions of the yohimbine congeners or of reserpine or propylene glycol were infused into the superfusion stream from 15 min before S_3 onwards. The rate of infusion was 16 µl/min. Drug effects on the evoked overflow of tritium were expressed as the ratio between the overflow (as per cent of tissue tritium) evoked by S_3 or S_4 and that evoked by S_2. In some cases, the drugs accelerated the basal outflow of tritium. The evoked overflow was not calculated whenever the outflow immediately before S_3 exceeded the outflow immediately before S_2 by more than 10%.

Individual ^{3}H-compounds in superfusate samples were determined by chromatography on alumina and Dowex 50WX4 columns according to Graefe et al. (1973). The method permits separation of the following fractions: ^{3}H-noradrenaline; ^{3}H-3,4-dihydroxyphenylglycol (DOPEG); ^{3}H-3,4-dihydroxymandelic acid (DOMA); ^{3}H-normetanephrine; and ^{3}H-O-methylated deaminated (OMDA) metabolites (sum of ^{3}H-3-methoxy-4-hydroxyphenylglycol and ^{3}H-3-methoxy-4-hydroxymandelic acid). Each fraction was corrected for cross contamination and recovery.

Antagonism to Exogenous Noradrenaline. Antagonist effects of the drugs against the contractile effect of noradrenaline were studied on artery strips that were suspended under 2 p of tension in an organ bath with 70 ml medium. After 1 h of equilibration during which the medium was changed every 15 min, a cumulative concentration-response curve for noradrenaline was determined. Concentrations were increased by the factor $\sqrt{10}$. The tissue was then washed for 1 h, the antagonist was added, and the tissue was incubated with the antagonist for a further 1 h with replacement of the medium every 15 min. Finally, a second concentration-response curve for noradrenaline was determined in the presence of the antagonist. EC_{50} values of noradrenaline were estimated by probit analysis and were used to calculate dose ratios. The regression of log (dose ratio − 1) on -log (antagonist concentration) was calculated, and the pA_2 value was determined as the point of intersection of the regression line with the concentration axis (Arunlakshana and Schild, 1959).

Drugs. Drug concentrations are final concentrations in the superfusion or incubation fluid. (–)-7-^{3}H-noradrenaline, specific activity 2.2 – 2.8 Ci/mmole, was periodically checked for purity by column chromatography. Yohimbine hydrochloride (Merck, Darmstadt, or Roth, Karlsruhe); rauwolscine hydrochloride and corynanthine (Roth, Karlsruhe); pseudoyohimbine (ICN Pharmaceuticals, Plainview, N.Y.); reserpine solution (Serpasil, Ciba-Geigy, Basel); cocaine hydrochloride (Merck, Darmstadt); corticosterone (Fluka, Buchs); (±)-propranolol hydrochloride (Rhein-Pharma, Heidelberg); (–)-noradrenaline hydrochloride (Höchst, Frankfurt am Main); clonidine hydrochloride (Boehringer, Ingelheim). β-Yohimbine, yohimbol and 3-epi-α-yohimbine were gifts from Professor H. Brunner, Ciba-Geigy, Basel. The identity of the yohimbine stereoisomers and of yohimbol was confirmed by infrared spectroscopy. No impurities were detected by thin layer chromatography. Alkaloid bases were dissolved in ascorbic acid solution, 2 moles ascorbic acid per mole base. Alkaloid hydrochlorides and clonidine hydrochloride were dissolved in water. The reserpine solution was diluted with water. For the cumulative concentration-response curves, noradrenaline hydrochloride was dissolved in 5.7 mM ascorbic acid solution. Corticosterone, dissolved in propylene glycol, and aqueous solutions of cocaine hydrochloride and propranolol hydrochloride were added directly to the superfusion or incubation fluid reservoir. The final concentration of the glycol was 6.8 mM.

Statistics. Standard statistical methods were used (Diem and Lentner, 1968). Unless otherwise stated, means ± S.E.M. are given. Significance of differences between means was calculated by *t*-test. *n*, number of experiments.

Results

The stereoisomers of yohimbine tested included the yohimbane derivatives β-yohimbine and corynanthine, the alloyohimbane derivative rauwolscine (= α-yohimbine), the pseudoyohimbane derivative pseudoyohimbine, and the 3-epialloyohimbane derivative 3-epi-α-yohimbine. Also examined was yohimbol which differs from yohimbine and its 16-epimer corynanthine by the absence of the 16-methoxycarbonyl group (for chemistry, see Hesse, 1964; Morrison, 1967; Lambert et al., 1978).

Effects on Noradrenaline Outflow and Metabolism and the Response to Stimulation

Rauwolscine, β-Yohimbine and Yohimbol. Experimental protocol and effect of rauwolscine are illustrated in Fig. 1. This strip was superfused with medium containing cocaine, corticosterone and propranolol in order to block amine uptake mechanisms and β-adrenoceptors, respectively. Upon electrical stimulation, the tissue contracted and the outflow of tritium was increased. In control experiments, contractions slightly increased from S_1 to S_4, whereas the evoked overflow of tritium remained approximately constant. Rauwolscine enhanced the contractile response and greatly augmented the stimulation-evoked overflow of tritium without changing the basal outflow. Responses to S_3 and S_4 were affected similarly. For further analysis, responses to S_4 were routinely evaluated, but an evaluation of responses to S_3 gave essentially the same results.

Concentration-response curves for rauwolscine are shown in Fig. 2. In the presence of cocaine, corticosterone and propranolol, as little as 10^{-9} M rauwolscine

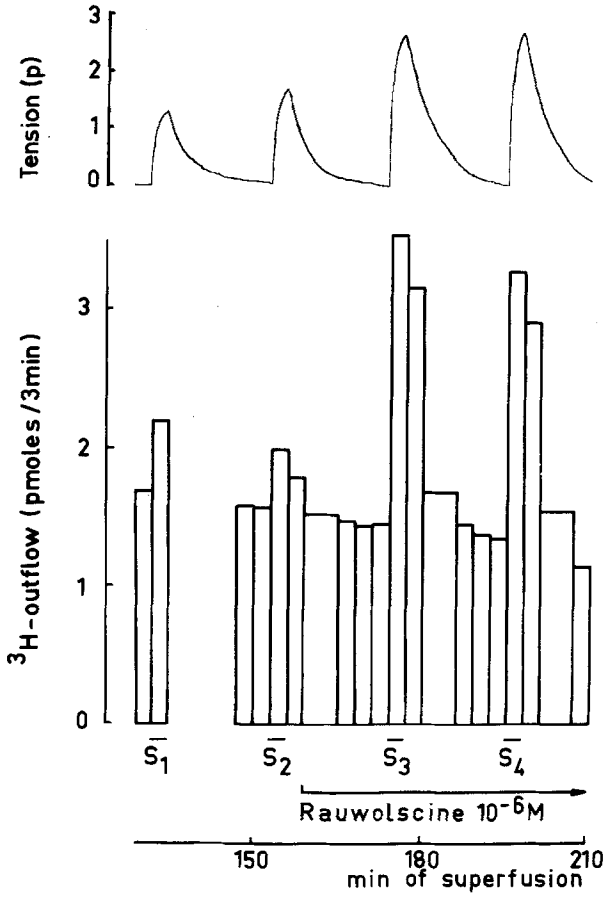

Fig. 1. Effect of rauwolscine 10^{-6} M on a pulmonary artery strip preincubated with ^{3}H-noradrenaline. After preincubation, the strip was superfused with ^{3}H-noradrenaline-free medium containing cocaine 3×10^{-5} M, corticosterone 4×10^{-5} M and propranolol 4×10^{-6} M. The superfusate was collected in 3- or 6-min samples. The strip was stimulated 4 times for 3 min each at 2 Hz ($S_1 - S_4$). The evoked overflow of tritium was 0.33 (S_2), 1.99 (S_3) and 2.01 % (S_4) of the tritium content of the tissue at the onset of the respective stimulation period

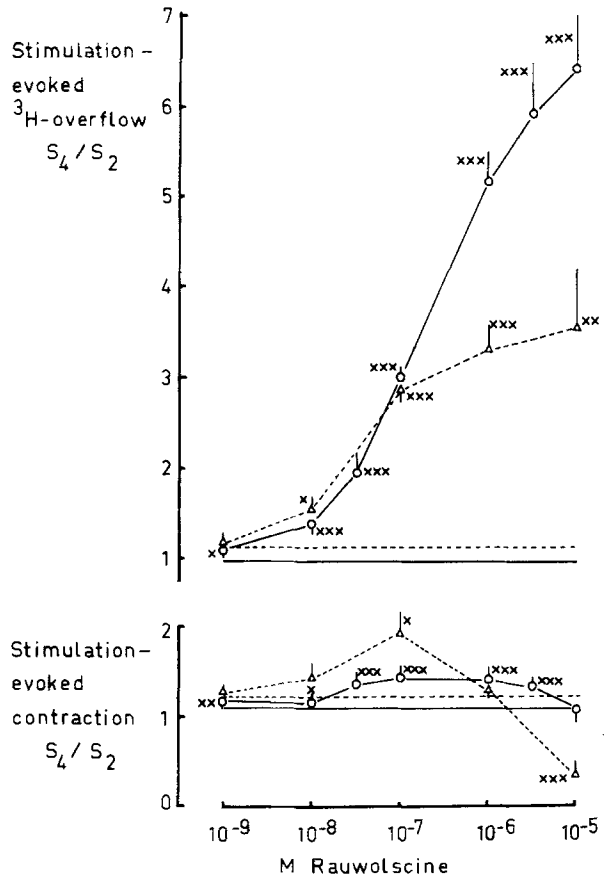

Fig. 2. Effect of rauwolscine on the response of pulmonary artery strips preincubated with ^{3}H-noradrenaline to sympathetic nerve stimulation. After preincubation, the strips were superfused either with medium containing cocaine, corticosterone and propranolol (○——○), or with medium that did not contain these drugs (△——△). Rauwolscine was added 15 min before S_3. Upper ordinate, ratio between the overflow of tritium evoked by S_4 and the overflow evoked by S_2. The overflow evoked by S_2 amounted to 0.46 ± 0.02 % ($n = 38$; with cocaine, corticosterone and propranolol) and 0.52 ± 0.05 % ($n = 23$; without these drugs) of the tritium content of the tissue, respectively. Lower ordinate, ratio between contractions evoked by S_4 and those evoked by S_2. Contractions evoked by S_2 amounted to 1.89 ± 0.11 p ($n = 37$; with cocaine, corticosterone and propranolol) and 0.81 ± 0.12 p ($n = 21$; without these drugs), respectively. Horizontal lines indicate mean ratios in control experiments with (——; $n = 9$) and without (----; $n = 4$) cocaine, corticosterone and propranolol. Each point is the mean of 3–6 experiments. Significant differences from controls: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Note that cocaine and corticosterone almost entirely prevent the uptake and degradation of noradrenaline released by nerve stimulation (Endo et al., 1977). The ensuing increase in biophase concentration of noradrenaline leads, firstly, to stronger α-adrenergic feedback inhibition of release, and this explains at least partly why cocaine and corticosterone failed to enhance the evoked overflow of total tritium (see S_2 values); and secondly, to the increase in contraction heights (see S_2 values)

marginally increased the stimulation-evoked overflow of tritium. At 10^{-5} M, the increase was more than 5-fold. Low concentrations enhanced the contractile response. Even 10^{-5} M rauwolscine failed to reduce the height of the contractions.

Somewhat different results were obtained when rauwolscine was administered in the absence of cocaine, corticosterone and propranolol. The maximal increase of the evoked overflow of tritium was smaller. Contractions were significantly enhanced only at one concentration, and were markedly reduced by rauwolscine 10^{-5} M.

The pattern of ^{3}H-compounds contributing to the outflow of total tritium is demonstrated in Fig. 3. These artery strips were superfused with cocaine-, corticosterone- and propranolol-free medium. As reported previously, most of the basal outflow consists of ^{3}H-OMDA metabolites and ^{3}H-DOPEG, whereas ^{3}H-noradrenaline and ^{3}H-normetanephrine are minor fractions; stimulation greatly increases the outflow of ^{3}H-noradrenaline; the outflow of ^{3}H-DOPEG, ^{3}H-

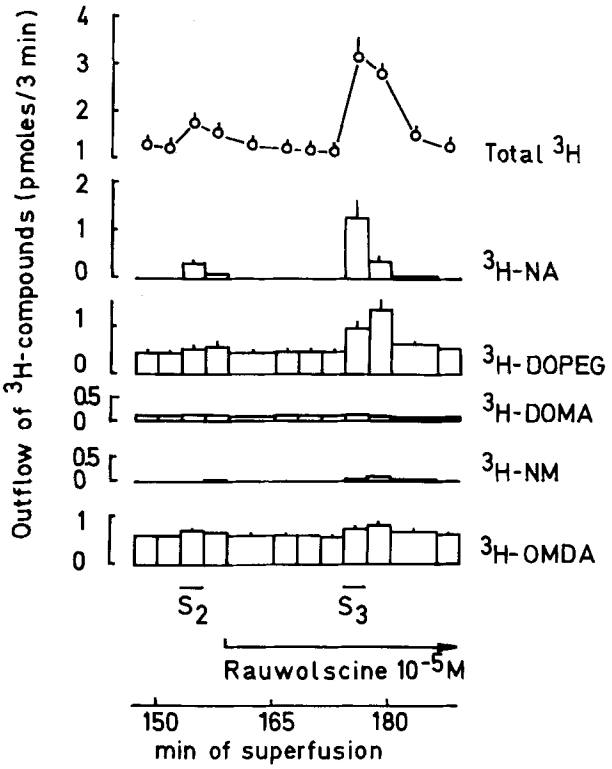

Fig. 3. Effect of rauwolscine 10^{-5} M on the outflow of ^{3}H-compounds from pulmonary artery strips preincubated with ^{3}H-noradrenaline. After preincubation, the strips were superfused with cocaine-, corticosterone- and propranolol-free medium. From top to bottom, outflow of total tritium, ^{3}H-noradrenaline (NA), ^{3}H-3,4-dihydroxyphenylglycol (DOPEG), ^{3}H-3,4-dihydroxymandelic acid (DOMA), ^{3}H-normetanephrine (NM) and ^{3}H-O-methylated deaminated (OMDA) metabolites. Means of 4 experiments

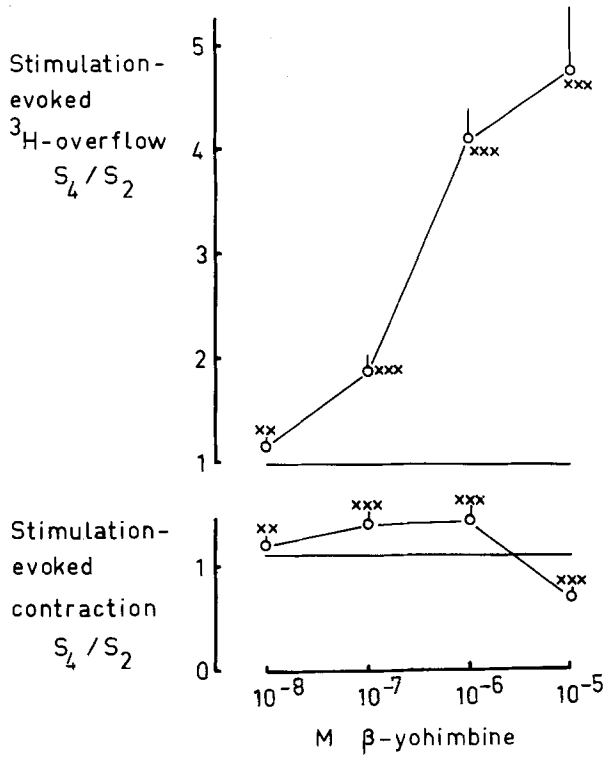

Fig. 4. Effect of β-yohimbine on the response of pulmonary artery strips preincubated with ^{3}H-noradrenaline to sympathetic nerve stimulation. After preincubation, the strips were superfused with medium containing cocaine, corticosterone and propranolol. β-Yohimbine was added 15 min before S_3. The overflow of tritium evoked by S_2 amounted to 0.44 ± 0.02 % of the tritium content of the tissue ($n = 30$). Contractions evoked by S_2 amounted to 1.46 ± 0.11 p ($n = 29$). Horizontal lines indicate mean S_4/S_2 ratios in control experiments ($n = 9$). Each point is the mean of 3–7 experiments. Significant differences from controls: ** $P < 0.01$; *** $P < 0.001$

normetanephrine and ^{3}H-OMDA metabolites is also increased but peaks *after* stimulation (Endo et al., 1977). Rauwolscine, even at the high concentration of 10^{-5} M, had virtually no effect on the basal outflow of any ^{3}H-compound. The increase in the evoked overflow of total tritium mainly reflected an increased overflow of ^{3}H-noradrenaline and ^{3}H-DOPEG.

The interaction between rauwolscine and clonidine was studied in strips superfused with cocaine, corticosterone and propranolol. Clonidine 10^{-5} M was infused into the superfusion stream from 18 min before S_1 onwards, and rauwolscine 3×10^{-8} M was added 15 min before S_3. Because at the concentration chosen clonidine almost abolished the overflow of tritium evoked by stimulation at 2 Hz, the frequency was increased to 4 Hz. Rauwolscine alone augmented the ratio between the overflow of tritium evoked by S_4 and the overflow evoked by S_2 to 1.91 ± 0.16 ($n = 3$). Clonidine prevented the facilitatory effect. In experiments with clonidine plus rauwolscine the ratio S_4/S_2 was 1.10 ± 0.08 ($n = 3$), i.e. close to the ratio in experiments with clonidine alone (1.11 ± 0.07; $n = 3$). This interaction closely resembles that between oxymetazoline and yohimbine (Starke et al., 1975a).

β-Yohimbine (Fig. 4) and yohimbol (Fig. 5) were tested only in the presence of cocaine, corticosterone and propranolol. They resembled rauwolscine, since low concentrations increased the evoked overflow of tritium and either enhanced (β-yohimbine) or did not change (yohimbol) the contractile response. High concentrations were needed to reduce stimulation-evoked contractions. β-Yohimbine did not affect, whereas yohimbol 10^{-5} M accelerated the basal outflow of tritium. Since, in the presence of yohimbol 10^{-5} M, the basal outflow before S_3 was 12 % higher than before S_2, the evoked overflow could not be calculated reliably.

Corynanthine. Corynanthine differed from rauwolscine, β-yohimbine and yohimbol in that higher concentrations were required to increase the evoked over-

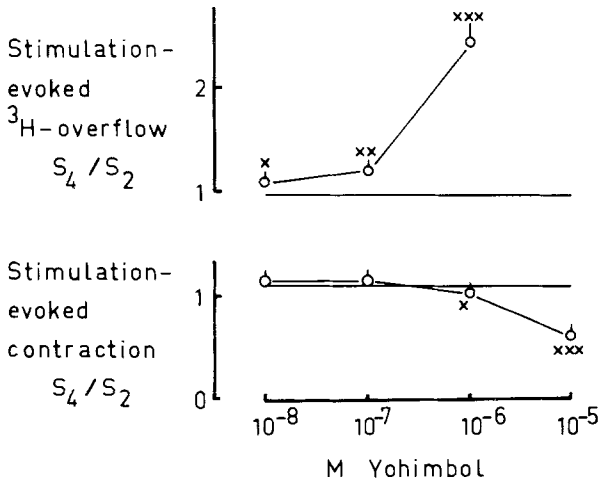

Fig. 5. Effect of yohimbol on the response of pulmonary artery strips preincubated with ^{3}H-noradrenaline to sympathetic nerve stimulation. After preincubation, the strips were superfused with medium containing cocaine, corticosterone and propranolol. Yohimbol was added 15 min before S_3. The overflow of tritium evoked by S_2 amounted to $0.45 \pm 0.03\%$ of the tritium content of the tissue ($n = 25$). Contractions evoked by S_2 amounted to 1.64 ± 0.09 p ($n = 28$). Horizontal lines indicate mean S_4/S_2 ratios in control experiments ($n = 9$). Each point is the mean of 4–6 experiments. Significant differences from controls: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

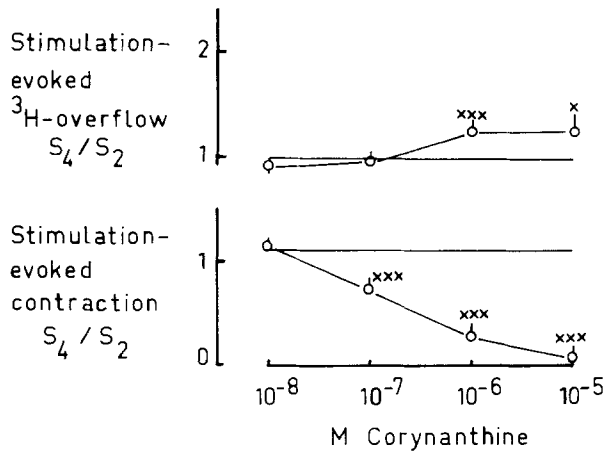

Fig. 6. Effect of corynanthine on the response of pulmonary artery strips preincubated with ^{3}H-noradrenaline to sympathetic nerve stimulation. After preincubation, the strips were superfused with medium containing cocaine, corticosterone and propranolol. Corynanthine was added 15 min before S_3. The overflow of tritium evoked by S_2 amounted to $0.44 \pm 0.02\%$ of the tritium content of the tissue ($n = 28$). Contractions evoked by S_2 amounted to 1.42 ± 0.11 p ($n = 28$). Horizontal lines indicate mean S_4/S_2 ratios in control experiments ($n = 9$). Each point is the mean of 3–5 experiments. Significant differences from controls: $^*P < 0.05$; $^{***}P < 0.001$

Table 1. Effect of pseudoyohimbine and 3-epi-α-yohimbine on pulmonary artery strips preincubated with ^{3}H-noradrenaline. After preincubation, the strips were superfused with medium containing cocaine, corticosterone and propranolol. Pseudoyohimbine or 3-epi-α-yohimbine was added 15 min before S_3. The basal outflow of tritium is calculated as the ratio between pmoles ^{3}H-outflow in the 3 min before S_3, and pmoles ^{3}H-outflow in the 3 min before S_2. The stimulation-evoked overflow and stimulation-evoked contractions are indicated as ratios between responses to S_3 or S_4 and responses to S_2

Drug added before S_3 (M)	Basal ^{3}H-outflow (before S_3/ before S_2)	Stimulation-evoked ^{3}H-overflow		Stimulation-evoked contraction		n
		S_3/S_2	S_4/S_2	S_3/S_2	S_4/S_2	
–	0.91 ± 0.01	1.04 ± 0.03	0.99 ± 0.02	1.07 ± 0.01	1.10 ± 0.01	9
Pseudoyohimbine 10^{-7}	0.86 ± 0.01*	1.04 ± 0.04	0.99 ± 0.04	1.05 ± 0.03	1.06 ± 0.05	3
Pseudoyohimbine 10^{-6}	0.97 ± 0.01**	1.09 ± 0.04	1.13 ± 0.10	1.07 ± 0.02	1.12 ± 0.03	6
Pseudoyohimbine 10^{-5}	1.28 ± 0.04***			0.75 ± 0.08***	0.67 ± 0.10***	6
3-epi-α-yohimbine 10^{-8}	0.90 ± 0.01	0.99 ± 0.04	0.97 ± 0.07	1.06 ± 0.02	1.15 ± 0.01	3
3-epi-α-yohimbine 10^{-7}	0.95 ± 0.01*	1.09 ± 0.04	1.11 ± 0.03**	1.12 ± 0.04	1.20 ± 0.05*	4
3-epi-α-yohimbine 10^{-6}	1.17 ± 0.08***			1.01 ± 0.02*	0.95 ± 0.07**	4
3-epi-α-yohimbine 10^{-5}	1.71 ± 0.02***			0.90 ± 0.07**	0.73 ± 0.12***	4

Significant differences from controls: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

flow of tritium than to depress stimulation-evoked contractions (Fig. 6). The increase of the evoked overflow was small. Basal tritium outflow remained unchanged.

Pseudoyohimbine and 3-epi-α-yohimbine. In contrast to the other alkaloids, pseudoyohimbine and 3-epi-α-yohimbine accelerated the basal outflow of tritium at relatively low concentrations, namely at 10^{-6} and 10^{-7} M, respectively (Table 1). At these concentrations, there was no clear-cut change in the response to stimulation. Higher concentrations reduced stimulation-evoked contractions, whereas the evoked overflow of tritium could not be calculated because of the large rise in basal outflow.

The influence of pseudoyohimbine, 3-epi-α-yohimbine and reserpine on the metabolic pattern of ^{3}H-noradrenaline was investigated in strips superfused with cocaine-, corticosterone- and propranolol-free medium. The effect of 3-epi-α-yohimbine 10^{-5} M is

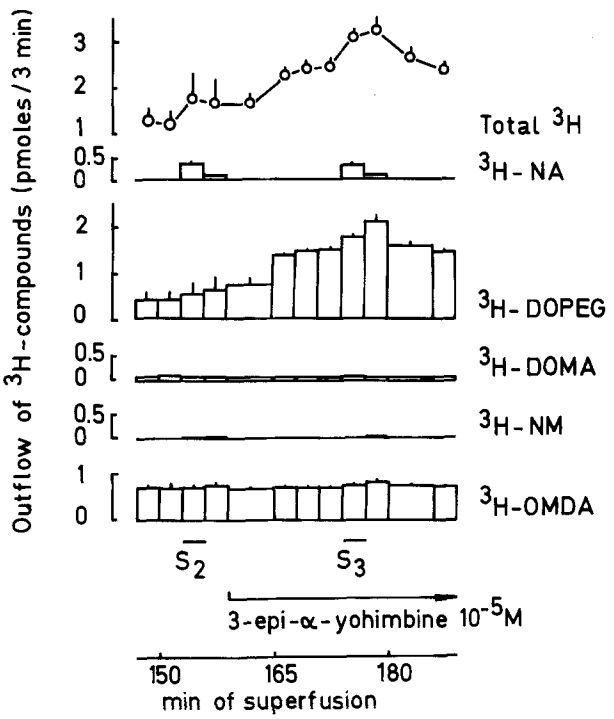

Fig. 7. Effect of 3-epi-α-yohimbine 10^{-5} M on the outflow of ^{3}H-compounds from pulmonary artery strips preincubated with ^{3}H-noradrenaline. Means of 4 experiments. See legend to Fig. 3 for further explanation

shown in Fig. 7. The drug accelerated the basal outflow of total tritium to about the same extent as in the presence of cocaine, corticosterone and propranolol. The effect was due to a selectively increased outflow of ^{3}H-DOPEG. The stimulation-evoked overflow of ^{3}H-noradrenaline at S_3 (in the presence of 3-epi-α-yohimbine) was similar to that observed at S_2 (before addition of the drug), just as it is in control experiments (not documented here; see Endo et al., 1977).

A selective increase in ^{3}H-DOPEG outflow was also observed with 10^{-6} M 3-epi-α-yohimbine as well as with pseudoyohimbine 10^{-5} M and reserpine 10^{-6} M. Moreover, in these experiments the evoked overflow of ^{3}H-noradrenaline was not changed either ($n = 2$ for each group; not shown).

Propylene Glycol. When corticosterone was added to the superfusion fluid, propylene glycol was used as solvent, final concentration 6.8 mM. Propylene glycol 14 mM accelerates the basal outflow of tritium from rabbit aortic rings preincubated with ^{3}H-noradrenaline (Schrold and Nedergaard, 1977). We tested its effect on pulmonary artery strips in experiments in which the glycol 6.8 mM (but not cocaine, corticosterone or propranolol) was present throughout superfusion. The strips were not stimulated electrically. The superfusate was collected in 10-min samples throughout, and the loss of tritium from the tissue with time was calculated. The desaturation curves thus obtained (Schrold and Nedergaard, 1977) were very similar for 5 strips treated with propylene glycol and for 5 control strips. After 210 min of superfusion the glycol-treated strips retained $59.8 \pm 1.8\%$ of their initial radioactivity; the corresponding control value was $63.6 \pm 1.9\%$ ($P > 0.1$). In additional experiments, the influence of propylene glycol 6.8 mM, added 15 min before S_3, on the response to stimulation was determined. It changed neither the evoked overflow of tritium nor the contractions ($n = 3$; not shown). Thus, propylene glycol changed none of the parameters measured in the experiments in which it was used as a solvent for corticosterone.

Antagonism to Exogenous Noradrenaline

The antagonism of the alkaloids to noradrenaline-induced contractions was studied on strips mounted in medium that contained cocaine, corticosterone and propranolol. When two successive concentration-response curves of noradrenaline were determined without addition of an antagonist, the EC_{50} values obtained were similar (mean value in first curves, 7.0×10^{-8} M; in second curves, 5.1×10^{-8} M); maximal contractions in the first curves amounted to 4.1 ± 0.4 p; those obtained in the second curves were greater, yielding a ratio of 1.30 ± 0.03 ($n = 7$). Yohimbine and its congeners were added before the second curves. They shifted them to the right without changing the ratio of the maxima. Plots according to Arunlakshana and Schild (1959) yielded straight lines with slopes not significantly different from -1 (Table 2). Corynanthine and yohimbine were the most potent antagonists, followed by β-yohimbine, yohimbol and rauwolscine. Pseudoyohimbine was a weak antagonist (Table 2). For lack of substance, 3-epi-α-yohimbine could be tested only in a few experiments at 3×10^{-5} M. Judged from the small shift of the concentration-response curve of noradrenaline, the pA_2 value was slightly lower than 5.

Discussion

The pulmonary artery of the rabbit has been repeatedly used for the study of presynaptic receptors (Starke et al., 1974; Borowski et al., 1977; Endo et al., 1977; Bevan, 1978; Constantine et al., 1978). Electrical stimulation, as employed here, selectively excites the postganglionic sympathetic neurones of the tissue. The evoked overflow of tritium is neural in origin. It consists of ^{3}H-noradrenaline that is released by a calcium-dependent mechanism and, in the absence of amine uptake inhibitors, of ^{3}H-metabolites formed

Table 2. Antagonism between yohimbine congeners and noradrenaline in pulmonary artery strips incubated in medium containing cocaine, corticosterone and propranolol. Two concentration-response curves for noradrenaline were determined on each strip. Antagonists were added 1 h before second curves. Results were evaluated to give coefficients of the regression of log(dose ratio −1) on −log(antagonist concentration) and pA_2 values

Antagonist	Regression coefficient (95% confidence interval)	pA_2 (95% confidence interval)	Number (and range) of antagonist concentrations tested	*n*
Yohimbine	−0.86 (−0.70 to −1.01)	6.56 (6.25 to 6.86)	4 ($3 \times 10^{-6} - 10^{-4}$ M)	11
Rauwolscine	−1.09 (−0.91 to −1.28)	5.89 (5.71 to 6.06)	4 ($3 \times 10^{-6} - 10^{-4}$ M)	11
β-Yohimbine	−0.89 (−0.68 to −1.11)	6.12 (5.83 to 6.41)	4 ($10^{-6} - 3 \times 10^{-5}$ M)	8
Yohimbol	−0.98 (−0.50 to −1.47)	6.00 (5.45 to 6.55)	3 ($3 \times 10^{-6} - 3 \times 10^{-5}$ M)	9
Corynanthine	−0.99 (−0.78 to −1.19)	6.60 (6.20 to 6.99)	4 ($3 \times 10^{-6} - 10^{-4}$ M)	12
Pseudoyohimbine	−0.82 (−0.53 to −1.11)	5.16 (4.89 to 5.43)	3 ($10^{-5} - 10^{-4}$ M)	13

from the released transmitter (Su and Bevan, 1970; Starke et al., 1974; Endo et al., 1977). Most of the present experiments were carried out in the presence of cocaine, corticosterone and propranolol in order to allow satisfactory determination of noradrenaline release and satisfactory characterization of α-adrenoceptors (see Furchgott, 1972; Borowski et al., 1977).

Our purpose was to compare pre- and postsynaptic α-adrenolytic actions of congeners of yohimbine. The drugs produced three effects. They inhibited contractions, enhanced the evoked overflow of tritium, and accelerated basal tritium outflow. The latter effect is probably unrelated to α-adrenoceptors (see below). The first question to be discussed is, then, whether the inhibition of contractions and the increase in the evoked overflow of tritium were in fact due to α-receptor blockade.

Although corynanthine and rauwolscine are traditionally considered to be α-adrenolytic drugs (Nickerson, 1949; Kohli et al., 1957), this has not been established definitely. In our experiments all alkaloids counteracted the contractile effect of noradrenaline. The antagonism was fully surmountable. Plots of log (dose ratio −1) against −log (antagonist concentration) gave straight lines with slopes close to −1 (Arunlakshana and Schild, 1959). These findings satisfy the criteria for competitive blockade of postsynaptic α-adrenoceptors (Furchgott, 1972). Moreover, since contractions elicited by nerve stimulation were inhibited by concentrations comparable to those that antagonized the effect of exogenous noradrenaline, it may be assumed that they were also reduced by *blockade of postsynaptic α-adrenoceptors.* (3-epi-α-yohimbine slightly diminished electrically evoked contractions at 10^{-6} M although it was a very poor antagonist of exogenous noradrenaline; the reason is not known, but it should be noted that the interaction with exogenous noradrenaline could not be studied in detail.)

Rauwolscine, β-yohimbine, yohimbol and corynanthine increased the evoked overflow of tritium in the presence of cocaine and corticosterone and, hence, by facilitation of noradrenaline release rather than by inhibition of amine uptake mechanisms (Borowski et al., 1977). Since there are numerous presynaptic receptor systems, sites other than α-adrenoceptors might be involved. However, it seems unlikely that the facilitation was due to interference with the presynaptic inhibitory effects of acetylcholine, prostaglandins or adenosine, because atropine, prostaglandin synthesis inhibitors and the adenosine antagonist theophylline produce no or only a marginal increase in noradrenaline release (Endo et al., 1977; Starke, unpublished results). Some yohimbine stereoisomers antagonize effects of serotonin (Lambert et al., 1978); an antiserotonin effect, however, can also be ruled out because serotonin does not affect noradrenaline release in the pulmonary artery (Endo et al., 1977). Moreover, β-adrenoceptors were blocked by propranolol. Finally, the α-receptor agonist clonidine at a presynaptically supramaximal concentration abolished the facilitatory effect of rauwolscine. Thus, there is reason to believe that at least the major part of the presynaptic effects also was α-adrenolytic in nature, i.e., due to *blockade of presynaptic α-adrenoceptors.*

The present study supplements a previous one in which we compared pre- and postsynaptic effects of nine α-adrenolytic drugs (Borowski et al., 1977). In that report, preferentially presynaptic antagonists such as yohimbine, preferentially postsynaptic antagonists such as clozapine and azapetine, and intermediate antagonists were distinguished. Three of the congeners of yohimbine, namely rauwolscine, β-yohimbine and yohimbol, fall into the preferentially presynaptic group as defined previously (Borowski et al., 1977); their presynaptic threshold concentration, i.e. the minimal concentration required for facilitation of release, was lower than their postsynaptic threshold concentration,

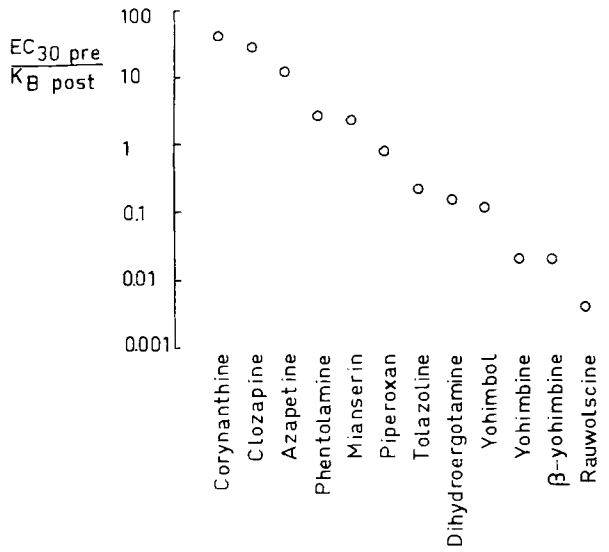

Fig. 8. Relation between pre- and postsynaptic effects of α-adrenolytic drugs in the pulmonary artery of the rabbit. $EC_{30\,pre}$ is the concentration that increases the stimulation-evoked overflow of tritium by 30%. $K_{B\,post}$ is the apparent dissociation constant of the antagonist-postsynaptic α-adrenoceptor complex ($= 10^{-pA_2}$). Results of experiments carried out in the presence of cocaine, corticosterone and propranolol in this and a previous investigation (Borowski et al., 1977) were evaluated. Corynanthine increased the evoked overflow of tritium by only 27% at 10^{-5} M, the highest concentration tested; this concentration was taken as the $EC_{30\,pre}$

i.e. the minimal concentration required for inhibition of stimulation-evoked contractions; moreover, their presynaptic threshold concentration was lower than the apparent dissociation constant of the antagonist-postsynaptic α-receptor complex ($K_{B\,post} = 10^{-pA_2}$). On the other hand, corynanthine clearly falls into the preferentially postsynaptic group, since the presynaptic threshold concentration exceeded the postsynaptic one and was also higher than $K_{B\,post}$. It should be noted that the difference between yohimbine, rauwolscine, β-yohimbine and yohimbol on the one hand and corynanthine on the other was mainly due to variation in presynaptic potency; the presynaptic threshold concentrations of the former drugs were only 1/100 or less of the presynaptic threshold concentration of corynanthine (10^{-6} M). Postsynaptic potencies differed much less; as can be seen from the pA_2 values of Table 2, the postsynaptic K_B values of the five alkaloids varied only 5 fold.

The relation between the pre- and postsynaptic potencies of all drugs of this and the previous study (except the irreversible antagonist phenoxybenzamine and except pseudoyohimbine and 3-epi-α-yohimbine which are discussed separately below) is illustrated in Fig. 8. $K_{B\,post}$ was taken to represent postsynaptic potency. The dissociation constant of the antagonist-presynaptic α-receptor complex cannot be determined from this kind of study (Starke et al., 1974). Instead, the concentration that increased the evoked overflow of tritium by 30% was chosen to represent presynaptic potency. Fig. 8 shows that two stereoisomers, namely β-yohimbine and rauwolscine, equal or even surpass yohimbine in preference for the presynaptic receptor. Yohimbine, β-yohimbine and rauwolscine also have in common that they enhance the contractile response to stimulation over a wide range of low concentrations. Yohimbol, dihydroergotamine, tolazoline and piperoxan, although also strong presynaptic α-adrenolytic drugs, fail to increase the postsynaptic response in the pulmonary artery. Figure 8 also shows that corynanthine surpasses clozapine and azapetine in preference for the postsynaptic α-receptor.

The ratios illustrated in Fig. 8 should not be overvalued. The $EC_{30\,pre}$ is an arbitrary measure of presynaptic potency, and calculations with, e.g., an $EC_{100\,pre}$ would have yielded different ratios, although the rank order of selectivity would have remained approximately the same. Moreover, the interpolation of the $EC_{30\,pre}$ values introduces a relatively large experimental error in particular when concentration-response curves are flat (e.g. Fig. 6). Notwithstanding such cautionary reflections, however, the now 10,000-fold range of relative potencies of antagonists again strongly supports the view that in a given tissue pre- and postsynaptic α-receptors may differ. Moreover, the results demonstrate that even chemically closely related drugs may greatly vary in their relative pre- and postsynaptic effects. It is remarkable that the extreme positions in Fig. 8 are occupied by two stereoisomers.

Receptors with the pharmacological properties of the presynaptic α-adrenoceptors of the rabbit pulmonary artery also occur outside noradrenergic terminal axons, as for instance on noradrenergic cell bodies in the locus coeruleus (Cedarbaum and Aghajanian, 1977) and in sympathetic ganglia (Brown and Caulfield, 1979), on cholinergic neurones (Drew, 1978; Wikberg, 1978), and on non-neuronal cells such as thrombocytes (Lasch and Jakobs, 1979). Berthelsen and Pettinger (1977) have suggested that these receptors, with the presynaptic α-receptor of the rabbit pulmonary artery as prototype, should be referred to as α_2-adrenoceptors, although this designation originally was used only for the α-adrenoceptors of noradrenergic axon terminals (Langer, 1974; cf. Starke and Langer, 1979). α_1-adrenoceptors (Langer, 1974) would then be receptors with the characteristics of the postsynaptic α-receptor of the rabbit pulmonary artery (Berthelsen and Pettinger, 1977). Their similarity in structure and some physical properties (Lambert et al., 1978) should make corynanthine and rauwolscine particularly useful tools for the subclassification of α-adrenoceptors.

Rauwolscine produced greater facilitation of noradrenaline release in the presence of cocaine, cortico-

sterone and propranolol than in their absence. Morevover, in the presence of the drugs the contractile response to stimulation was more resistant to depression by rauwolscine. The reason may be the increase in biophase concentration of noradrenaline brought about by cocaine and corticosterone (Endo et al., 1977). The α-adrenergic feedback inhibition of release will then be stronger and, consequently, the facilitatory effect of rauwolscine (at concentrations that are high with respect to the presynaptic receptors) greater; an analogous finding is that cocaine *decreases* the presynaptic *inhibitory* effect of the α-adrenergic *agonist* clonidine (Starke and Altmann, 1973). On the other hand, the high concentration of noradrenaline caused by the uptake inhibitors plus rauwolscine may almost saturate the postsynaptic α-adrenoceptors so that rauwolscine (at concentrations that are low with respect to the postsynaptic receptors) cannot effectively compete with the transmitter.

Pseudoyohimbine and 3-epi-α-yohimbine were at best weak antagonists at both the pre- and the postsynaptic α-adrenoceptors. In further contrast to the other alkaloids, they accelerated the basal outflow of ^{3}H-DOPEG at relatively low concentrations. A similar increase in DOPEG outflow is produced by high concentrations of many agents including α-adrenolytic drugs (Adler-Graschinsky et al., 1972; Graefe et al., 1973; Borowski et al., 1977). It is probably unrelated to α-receptor blockade and reflects an impairment of the vesicular storage of noradrenaline as typically elicited by reserpine-like substances (Adler-Graschinsky et al., 1972) including reserpine itself (present results).

Can the different pharmacological profiles of the congeners of yohimbine be related to their structure (for chemistry, see Hesse, 1964; Morrison, 1967; Lambert et al., 1978)? Pseudoyohimbine and 3-epi-α-yohimbine share with reserpine not only a prominent granular site of action but also the 3α-configuration, whereas all other alkaloids have the β-configuration at $C_{(3)}$. The 3α-configuration, which makes the nucleus of the molecules angular, may favour reserpine-like activity, whereas the 3β-configuration with a relatively planar ring system may favour α-adrenolytic activity. Within the group of 3β-configurated compounds, the alkaloids differ more in their presynaptic than their postsynaptic α-adrenolytic potency. Molecular models show that rauwolscine, β-yohimbine, yohimbine and yohimbol possess a relatively flat upper surface of the molecule without projecting substituents. Furthermore, elimination of the 16-methoxycarbonyl group of yohimbine to give yohimbol does not greatly reduce presynaptic potency indicating that the group is not necessary for a strong presynaptic effect. On the other hand, epimerisation at $C_{(16)}$ to give corynanthine results in a pronounced loss of presynaptic potency. In corynanthine, the 16-methoxycarbonyl group projects perpendicularly upwards from the ring system. These considerations suggest that a flat upper surface of the molecule is a prerequisite for high affinity to the presynaptic receptor, and that disruption of the surface by a large polar substituent seriously impairs the presynaptic effect. As mentioned above, affinity to the postsynaptic α-adrenoceptor appears to be less sensitive to structural changes in this series of compounds.

In the scanty pharmacological work on yohimbine stereoisomers published, some parallels to the present results are of interest. Rauwolscine, β-yohimbine, yohimbine, pseudoyohimbine and corynanthine are, with potency decreasing in that order, antagonists of serotonin on fundus strips of the rat stomach (Lambert et al., 1978). Yohimbine and β-yohimbine increase, whereas corynanthine decreases, the toxicity of amphetamine in mice (Lambert and Lang, 1977). Systemically injected rauwolscine and β-yohimbine, like yohimbine, increase the blood pressure and cause behavioural excitation in conscious dogs, whereas pseudoyohimbine and corynanthine do not produce these effects (mentioned by Lambert et al., 1978). Corynanthine also fails to mimic some neurochemical and behavioural effects of yohimbine in rats (Papeschi et al., 1971). More detailed in vivo studies of the neuropharmacology of yohimbine stereoisomers might help to clarify the role of α_1-adrenoceptors, α_2-adrenoceptors and possibly serotonin receptors in the central nervous system.

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