Identification of Vascular Postsynaptic $\alpha_1$- and $\alpha_2$-Adrenoceptors in Man

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SUMMARY. We studied postsynaptic $\alpha_1$-adrenoceptors in human blood vessels by measuring the influence on forearm blood flow induced by intra-arterial infusions of selective $\alpha_1$- and $\alpha_2$-adrenoceptor agonists (methoxamine, B-HT 933, clonidine and guanfacine) and antagonists (doxazosin and yohimbine). The studies were done in healthy volunteers, and forearm blood flow was measured by plethysmography. All agonists produced a significant and dose-dependent vasoconstriction. The effect of B-HT 933 was completely abolished by the concomitant infusion of yohimbine, whereas it was hardly influenced by doxazosin. The effect of methoxamine was prevented by doxazosin and little influenced by yohimbine. The vasoconstriction by clonidine and guanfacine was partially prevented by both doxazosin and yohimbine. The single intra-arterial infusion of yohimbine, as well as doxazosin, resulted in vasodilation. These findings provide strong evidence for the existence of postsynaptic $\alpha_1$- as well as $\alpha_2$-adrenoceptors, both mediating vasoconstriction and contributing to basal vascular tone. The (patho-)physiological significance of this subdivision of $\alpha$-adrenoceptors remains to be elucidated. (Circ Res 54: 447-452, 1984)

ON the basis of relative activities and affinities of agonists and antagonists, $\alpha$-adrenoceptors can be subdivided into $\alpha_1$- and $\alpha_2$-subtypes (Berthelsen and Pettinger, 1977; Wikberg, 1979; Langer, 1980; Timmermans and van Zwieten, 1981, 1982; Starke, 1981; McGrath, 1982). Originally, it was thought that $\alpha_2$-adrenoceptors were exclusively located presynaptically, but at present there is ample evidence for the existence of $\alpha_2$-adrenoceptors outside noradrenergic terminal axons, on some organelles lacking synapses, and even at postsynaptic sites (Berthelsen and Pettinger, 1977; Starke, 1977; Wikberg, 1979; Timmermans and van Zwieten, 1982). Indeed, postsynaptic $\alpha_2$-adrenoceptors have been identified by in vivo experiments in vascular smooth muscle of various animal species including the rat (Drew and Whiting, 1979; Docherty et al., 1979; Timmermans and van Zwieten, 1980a; Hicks and Cannon, 1980), rabbit (van Meel et al., 1981; Hamilton and Reid, 1982), dog (Constantine et al., 1980; Langer et al., 1981), and cat (Drew and Whiting, 1979; Timmermans et al., 1983).

In man, the contribution of postsynaptic $\alpha$-adrenoceptors to basal vascular tone has been studied with the nonselective $\alpha$-antagonists phenoxybenzamine, and phentolamine (Lowe and Robinson, 1964; Abboud et al., 1968; Kiowski et al., 1981). Evidence for more than one type of postsynaptic $\alpha$-adrenoceptors in vascular smooth muscle with use of selective $\alpha$-antagonists, has been limited to in vitro experiments with strips of arteries and veins (Moulds and Jauernig, 1977; Jauernig et al., 1978; Stevens and Moulds, 1981). We have presented preliminary evidence for a postsynaptic $\alpha_2$-adrenoceptor in the vasculature of the forearm by showing dose-dependent vasoconstriction evoked by the intra-arterial infusion of the selective $\alpha_2$-agonist B-HT 933, and dose-dependent vasodilation upon intra-arterial infusion of the $\alpha_2$-antagonist yohimbine (Van Brummelen et al., 1983). Experiments with the selective $\alpha_2$ antagonist, RX79104, in healthy volunteers by Elliott and Reid (1983) were also consistent with the existence of a postsynaptic $\alpha_2$-adrenoceptor on vascular smooth muscle.

The present study was designed in order to substantiate further the existence of postsynaptic $\alpha_1$- and $\alpha_2$-adrenoceptors in human blood vessels by measuring the influence on forearm blood flow of single and combined infusions of $\alpha_1$- and $\alpha_2$-adrenoceptor agonists and antagonists in healthy volunteers. In these experiments, the highly selective agonists methoxamine ($\alpha_1$) (Starke et al., 1975a; Van Meel et al., 1981) and B-HT 933 ($\alpha_2$) (Kobinger and Pichler, 1977; Timmermans and van Zwieten, 1980a, 1980b; Van Meel et al., 1981; Rubin et al., 1982) were used, as well as the less selective agonists, clonidine ($\alpha_2 > \alpha_1$) (Starke et al., 1975a; Berthelsen and Pettinger, 1977; Kobinger, 1978; Timmermans and van Zwieten, 1980a) and guanfacine ($\alpha_2 > \alpha_1$) (Kobinger, 1978; Timmermans et al., 1979; Jerie, 1980; Reid et al., 1980, 1983). Doxazosin ($\alpha_1$) (Timmermans et al., 1980) and yohimbine ($\alpha_2$)
Methods

Drugs and Solutions

The following drugs were infused intra-arterially: B-HT 933-2HCl (azepexole, Boehringer Ingelheim), methoxamine-HCl (Wellcome), clonidine-HCl (Boehringer Ingelheim), guanfacine-HCl (Sandoz), doxazosin (UK-33,274, Pfizer), and yohimbine-HCl (tested according to Ph. Helv.). The drugs were dissolved in 5% dextrose (wt/vol). All solutions were prepared on the day of the study and kept at 4°C until used.

Study Protocol

Infusions of Methoxamine and B-HT 933 with Saline, Yohimbine, and Doxazosin

In six volunteers, methoxamine and B-HT 933 were infused in random order, in the presence of a constant infusion of saline (0.4 ml/min). The cumulative doses used were: methoxamine, 0.04, 0.08, and 0.20 µg/kg per min, and B-HT 933, 0.20, 0.40, and 1.00 µg/kg per min. Each dose was given for 3 minutes. Then the infusions of methoxamine and B-HT 933 were repeated in the presence of yohimbine (1.0 µg/kg per min; infusion rate 0.4 ml/min). Finally, the infusions of methoxamine and B-HT 933 were repeated in the presence of doxazosin (0.1 µg/kg per min, infusion rate 0.4 ml/min). The order of infusion of the antagonists was not randomized because of the much longer duration of vasodilation after infusion of doxazosin, compared with yohimbine (unpublished observations).

The infusions of saline, yohimbine, and doxazosin were started 3 minutes before the cumulative dose infusions. FBF was measured immediately before starting the infusions, during the last 90 seconds of the single infusions of saline, yohimbine and doxazosin, and, subsequently, during the last 90 seconds of each dose step of the agonists.

Infusions of Clonidine and Guanfacine with Saline, Yohimbine, and Doxazosin

In six volunteers, clonidine and guanfacine were infused in the presence of a constant infusion of saline. The cumulative doses used were: clonidine, 0.009, 0.024, and 0.060 µg/kg per min, and guanfacine, 0.04, 0.08, and 0.20 µg/kg per min. Each dose was given for 3 minutes. The infusions of clonidine and guanfacine were repeated, first in the presence of yohimbine (0.5 µg/kg per min) and then in the presence of doxazosin (0.1 µg/kg per min). FBF was measured at the same points as mentioned in the previous section.

Dose Dependency of the α2-Blocking Effect of Yohimbine

In order to investigate the dose dependency of the yohimbine effect, we infused clonidine in two volunteers, first in the presence of saline and then together with yohimbine, 1.0 µg/kg per min. In these two subjects, B-HT 933 was also infused, first in the presence of saline and, subsequently, together with yohimbine, 0.5 µg/kg per min. Also, in these experiments, the infusions of saline and yohimbine were started 3 minutes before the cumulative dose infusions. FBF was measured at the same points as mentioned before.

Statistical Analysis

Results are given as the mean ± 1 sd. Two-way analysis of variance and Student's t-test for paired observations were used for statistical evaluation. P values less than 0.05 were regarded as significant.

Results

In all experiments, changes in HR and ia BP were small and inconsistent, excluding important systemic hemodynamic effects of the drugs in the doses used (Table 1).

Infusions of B-HT 933 and Methoxamine with Saline, Yohimbine, and Doxazosin

FBF was unaltered during infusion of saline, but it increased significantly by 81 ± 38% during infusion of yohimbine, 1.0 µg/kg per min (t = 5.04, P < 0.01, n = 5), and by 42 ± 35% during doxazosin, 0.1 µg/kg per min (t = 2.78, P < 0.05, n = 5).

Percent changes in FBF during the combined infusions are depicted in Figures 1 and 2. B-HT 933,
together with saline, produced a dose-dependent decrease in FBF (F = 33.41, P < 0.001, n = 6). This decrease in FBF was not significantly influenced by doxazosin (F = 1.12, P > 0.05, n = 5), whereas it was completely prevented by the simultaneous infusion of yohimbine (F = 78.21, P < 0.001, n = 6). Methoxamine, together with saline, produced a dose-dependent decrease in FBF (F = 40.05, P < 0.001, n = 6) which was not significantly influenced by yohimbine (F = 4.85, P > 0.05, n = 6). During infusion of doxazosin, the effect of methoxamine on FBF was completely abolished (F = 25.99, P < 0.005, n = 4).

Infusions of Clonidine and Guanfacine with Saline, Yohimbine, and Doxazosin

Yohimbine, 0.5 μg/kg per min, increased FBF significantly by 98 ± 63% (t = 5.10, P < 0.001, n = 6) and doxazosin 0.1 μg/kg per min increased FBF by 86 ± 38% (t = 4.60, P < 0.02, n = 6). Percent changes in FBF during the combined infusions are shown in Figures 3 and 4. Clonidine with saline produced a dose-dependent decrease in FBF (F = 4.43, P < 0.05, n = 6) which was not significantly altered by yohimbine (F = 0.16, P > 0.05, n = 6), but it was significantly reduced by doxazosin (F = 4.25, P < 0.05, n = 6). Guanfacine with saline reduced FBF in a dose-dependent way (F = 11.98, P < 0.005, n = 5). This decrease in FBF was significantly reduced by yohimbine (F = 6.65,
Dose Dependency of the \( \alpha_2 \)-Blocking Effect of Yohimbine

In two subjects it was shown, in a limited preliminary study, that the influence of clonidine on FBF was markedly reduced by yohimbine in a dose of 1.0 \( \mu \text{g/kg per min} \) (Fig. 5). In one of these subjects, the effect of B-HT 933 was partially prevented by yohimbine, 0.5 \( \mu \text{g/kg per min} \).

**Discussion**

Measurement of changes in FBF in response to local intra-arterial infusion of drugs has often been used for the in vivo study of drug-receptor interactions in humans (Roddie and Wallace, 1979). In this model, local effects are seen long before systemic hemodynamic changes occur, and, hence, baroreflex alterations are induced. In the present study, all changes in FBF were observed in the absence of changes in heart rate or blood pressure, indicating that the former results from direct drug effects on vascular tone. It should be noted, however, that results obtained in this particular vascular bed cannot be automatically extrapolated to other parts of the circulation.

Our results provide strong evidence for the existence of a postsynaptic \( \alpha_1 \)- as well as \( \alpha_2 \)-adrenoceptor in the human forearm vascular bed. Until now, evidence for the presence of the classical \( \alpha_1 \)-adrenoceptor in the vasculature of the human forearm was limited to the demonstration by Amann and co-workers (Amann et al., 1981) of vasodilation upon the intra-arterial infusion of the selective \( \alpha_1 \)-antagonist, prazosin. In the present study, we have verified these results with another selective \( \alpha_1 \)-antagonist, doxazosin. Moreover, we have shown that doxazosin in the dose used did not influence \( \alpha_2 \)-adrenoceptor-mediated vasoconstriction and that the intra-arterial infusion of the \( \alpha_1 \)-agonist, methoxamine, produces dose-dependent vasoconstriction. It was confirmed in the present study that intra-arterial infusion of the specific \( \alpha_2 \)-agonist, B-HT 933, resulted in a dose-dependent vasoconstriction, and that infusion of yohimbine caused vasodilation (van Brummelen et al., 1983). These findings suggest the presence of a postsynaptic \( \alpha_2 \)-adrenoceptor that mediates vasoconstriction and contributes to basal vascular tone. Additional and stronger evidence for this \( \alpha_2 \)-adrenoceptor was provided by the finding that B-HT 933-induced vasoconstriction was completely abolished by yohimbine but was not influenced by doxazosin. In the same subjects, we have shown that yohimbine, in the dose used, had no appreciable \( \alpha_1 \)-adrenoceptor-blocking effect.

In similar experiments, we have studied the \( \alpha \)- and \( \alpha_2 \)-agonist activity of clonidine and guanfacine, two antihypertensive drugs with an established central mode of action and mixed \( \alpha \)-adrenoceptor-stimulating effects (Kobinger, 1978; Timmermans et al., 1979; Jerie, 1980; Reid et al., 1980, 1983). A dose-dependent vasoconstrictor (i.e., postsynaptic) effect was shown for both drugs. As expected, this effect was not caused exclusively by \( \alpha \)-stimulation, since it was also significantly inhibited by doxazosin. In fact, an \( \alpha_2 \)-component for clonidine was demonstrated only after the dose of yohimbine was increased to 1.0 \( \mu \text{g/kg per min} \).

From our data, no conclusion can be drawn with respect to the relative contribution of the postsynaptic \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptor to basal vascular tone, as we did not perform dose-response curves for both antagonists. Moreover, it is likely that maximal vasodilation by intra-arterial infusion of either doxazosin or yohimbine would require doses which cause systemic hemodynamic effects, so that changes in forearm blood flow would be difficult to interpret.
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Despite the fact that B-HT 933 (azepexole), guanfacine, and clonidine are antihypertensive drugs, they all cause vasoconstriction after local administration. On the other hand, yohimbine, known to increase blood pressure (Nickerson and Collier, 1970) when administered systemically, causes vasodilation when infused intra-arterially. These opposite effects can be explained by a preferential action of these drugs on postsynaptic $\alpha_2$-adrenoceptors in the central nervous system (Kobinger, 1978; Reid et al., 1983; Van Zwieten et al., 1983), stimulation of which leads to activation of an (hypothetical) inhibitory neuron, resulting in diminished sympathetic activity, thus causing a hypotensive effect. However, involvement of presynaptic $\alpha_2$-adrenoceptors cannot be excluded completely.

On the basis of the results of the present study, there is little doubt that in man, vascular postsynaptic $\alpha$-adrenoceptors can be divided into $\alpha_1$ and $\alpha_2$ subtypes and that the vasoconstriction mediated by these receptors contributes to basal vascular tone.

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FIGURE 5. Percentage change in FBF during ia infusion of three cumulative doses of clonidine in the presence of saline and yohimbine (1.0 $\mu$g/kg per min) and during ia infusion of three cumulative doses of B-HT 933 in the presence of saline and yohimbine (0.5 $\mu$g/kg per min).

Although the present study does not provide any new information, it should be noted that the results presented here support the hypothesis that $\alpha_2$-adrenoceptors play a significant role in basal vascular tone.

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