

Basic Sciences In Medicine

CONTRACTIONS DUE TO α -ADRENOCEPTOR AGONISTS ARE MEDIATED BY α_1 -ADRENOCEPTORS IN RAT CAROTID ARTERY

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ABSTRACT

Some large vessels have a mixed functional population of postjunctional α_1 - and α_2 -adrenoceptors. The purpose of the work presented here was to investigate the population of postjunctional α -adrenoceptors in the rat isolated common carotid artery. Male Wistar rats were killed by overdose with pentobarbitone sodium, after which the left and right common carotid arteries were removed. Rings of arteries 3-4 mm in length were cut from each vessel and then mounted in 10 mL isolated organ bath, bathed in Krebs maintained at 37°C and gassed with 95% O₂ plus 5% CO₂. The preparations were allowed to equilibrate for an hour. When antagonists were used, the preparations were incubated for at least 45 minutes with the drugs prior to the onset of a second CCRC. The current study focused on the possibility of postjunctional α_2 -adrenoceptors that could influence adrenergic system-mediated vascular α -adrenoceptor responsiveness in this cephalic artery. The dominance of α_1 -adrenoceptors is shown by the high sensitivity of noradrenaline or phenylephrine to prazosin and the ineffectiveness of rauwolscine, except in non-selective concentrations. UK-14304 produced contractions and it is theoretically possible that UK-14304 exerts its actions through combined α_1 and α_2 activation, but the effectiveness of prazosin and the ineffectiveness of rauwolscine, except in non-selective concentrations, shows that even this effect is mediated through α_1 -adrenoceptors. Thus we suggest that the population of postjunctional α -adrenoceptors mediating contraction of smooth muscle in the rat carotid artery is predominantly of the α_1 type.

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INTRODUCTION

Oliver & Schafer¹ demonstrated that injection of extracts of the adrenal gland caused a rise in arterial pressure *in vivo*. The initial view about adrenoceptors held by Elliot² was that adrenaline was the most likely mediator of sympathetic neurotransmission. The α -adrenoceptors are intrinsic membrane

glycoproteins that mediate a variety of important sympathetic nervous system responses. They mediate a variety of functions and have been of major interest for many years as targets for drug action, and implicated in many human diseases. In 1948 Ahlquist³ concluded that the differences in potency orders could only be explained by assuming differences in the receptors. Therefore he classified adrenoceptors

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initially into α and β subtypes. Effector cells with α -adrenoceptors had a high sensitivity to adrenaline and noradrenaline but were practically insensitive to isoprenaline.³ The discovery of presynaptic α -adrenoceptors and their role in the modulation of noradrenergic neurotransmission provided the stimulus for the subclassification of α -adrenoceptors. This subclassification developed as a result of the pharmacological differences between presynaptic α_2 -adrenoceptors that mediate inhibition of the release of noradrenaline from sympathetic nerve terminal and postsynaptic α_1 -adrenoceptors. Differences in relative potencies of agonists which stimulate and antagonists which block these receptors have led to the conclusion that postsynaptic receptors are qualitatively different from presynaptic receptors.⁴ Subsequent work in this field, using a number of selective agonist and antagonist drugs, has confirmed the existence of α_1 - and α_2 -adrenoceptors postsynaptically.⁵ In general, α -adrenoceptors of the α_1 type are most effectively activated by phenylephrine and antagonized by prazosin, selective for the α_1 -adrenoceptors.⁶ Responses to α -adrenoceptor agonists in the vast majority of isolated vascular preparations, particularly arterial vessels, have been shown to be sensitive to prazosin.⁷ The rauwolfia alkaloids, rauwolscine and yohimbine were originally shown to be highly selective α_2 -antagonists.⁸ The α -adrenoceptors classified as α_2 are preferentially stimulated by clonidine and inhibited by rauwolscine. Although clonidine has some degree of selectivity for α_2 -adrenoceptors, it is not a full agonist and has partial agonist effects on the α_1 -adrenoceptor subtype.⁹ UK-14304 is a full agonist at α_2 -adrenoceptors in various pharmacological preparations.¹⁰ BHT-920 has been also used as a selective α_2 -adrenoceptor agonist.¹¹ Noradrenaline stimulation of both postjunctional α_1 - and α_2 -adrenoceptors can be demonstrated in pithed rabbits, using the sequential administration of the antagonists prazosin and rauwolscine, the combination of which produces a greater effect than either antagonist per se.⁷ We evaluated the effect of different agonists and antagonists of α -adrenoceptors on the carotid artery to determine whether this artery contains functional postjunctional α_2 -adrenoceptors capable of promoting vascular responsiveness of the adrenergic system.

MATERIALS AND METHODS

Common carotid arteries (700 μ m in lumen diameter) were obtained from male Wistar rats, weighing 320-400g, which were killed by overdose with pentobarbitone sodium (i.p. injection). Although the carotid artery is smaller than the aorta, a pair of common carotid arteries were easily dissected out and were placed in cold, oxygenated modified Krebs-Henselite solution (Krebs). The arteries were cleaned of any extraneous connective tissue using fine scissors. Each preparation was cut transversely into 3-4mm rings and suspended between thick wire supports. During the prepara-

tion of the arterial ring segments, any contact with the luminal surfaces was avoided to preserve endothelial integrity. Each ring was suspended horizontally by means of two stainless-steel L-shaped hooks carefully passed through the lumen. The upper support was connected by cotton to an isometric transducer while the lower support was connected to a glass tissue holder. The arterial rings were mounted in 10 mL isolated organ bath, bathed in Krebs maintained at 37°C and gassed with 95% O₂ plus 5% CO₂. The rings were then placed under resting tension at 2.5-3g for each group of arterial rings of the carotid artery. Isometric contractions were measured by a Grass FT03 transducer connected to a Linseis (TYP 7208) pen recorder. In all experiments, tissues were left to equilibrate for a 60 min period, during which time the tension was re-adjusted to a set value which was maintained constant throughout the rest of the experimental day. Each preparation was then exposed to NA (1 μ M) and allowed to contract for 5-10 min. This first contraction to an agonist minimizes changes in the sensitivity of preparations to further addition of agonists. Following complete washout, an additional one hour equilibration period was allowed before commencement of any other experimental procedure. Cumulative concentration-response curves (CCRC) were constructed in a cumulative manner by increasing the concentration of the agonists in half-log increments. When responses to agonists were not maintained, addition of the next concentration was made as close to the peak as possible. An initial control CCRC, to any given agonist, was obtained in each preparation. Following attainment of the maximal control contraction, preparations were washed until complete relaxation was effected. The preparations were then left for a further period of 45-60 min before re-exposure to the agonist. When the competitive antagonists like prazosin and rauwolscine were used, the preparations were incubated at least for 45 minutes with the drugs prior to the onset of a second CCRC. Results are expressed as mean \pm standard error of mean. Comparisons between two groups were performed using the paired or unpaired Student's t-test. Comparisons among several groups were performed using one-way analysis of variance. A value of $p < 0.05$ was taken statistically significant.

Table I. List of pA₂ values with the slopes of the Schild plots (with 95% confidence limits) for α -adrenoceptor antagonists against responses to noradrenaline in the rat isolated common carotid artery.

Antagonist	pA ₂	Slope
Rauwolscine	6.9 (6.63-7.2)	0.72 (0.61-0.84)
Prazosin	10.05 (9.8-10.32)	1.04 (0.94-1.1)

pA₂ values were determined from a regression analysis of the logarithm of dose ratio-1 against the negative logarithm of the molar concentration of the antagonist.

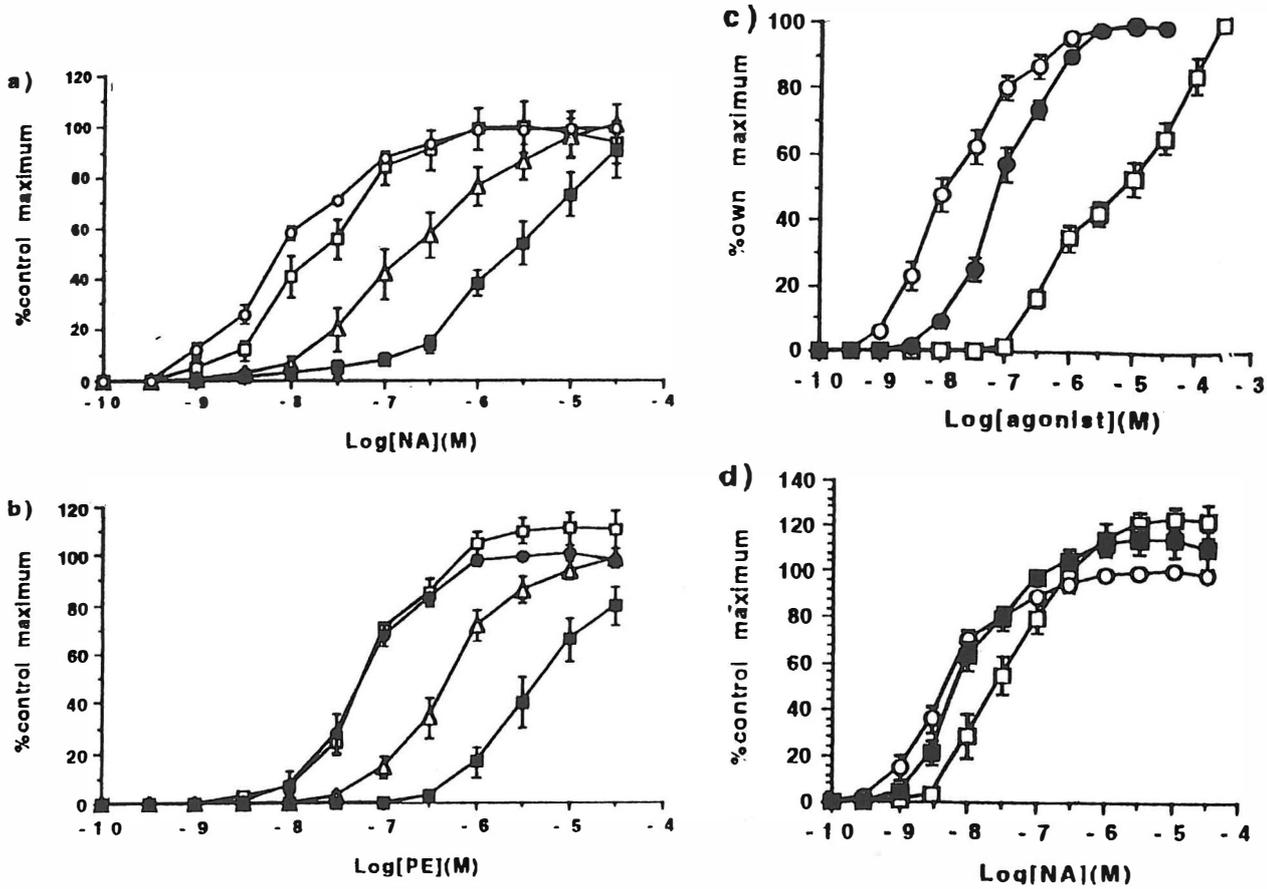


Fig. 1. Effects of selective α_1 -adrenoceptor antagonist prazosin 0.1 mM (\square), 1 nM (Δ) or 10 nM (\blacksquare) on control responses to a) noradrenaline (\circ) b) phenylephrine (\bullet) in the rat isolated common carotid artery. Responses are expressed as % of the maximum response of the control cumulative concentration-response curves (CCRC) to noradrenaline or phenylephrine in the absence of antagonist. c) CCRC to α -adrenoceptor agonists: noradrenaline (\circ), phenylephrine (\bullet) and UK-14304 (\square) in the rat isolated common carotid artery. Results are expressed as % of the maximum response of each individual CCRC to agonist. d) Effects of selective α_2 -adrenoceptor antagonist rauwolscine 0.1 μ M (\blacksquare) or 1 μ M (\square) on control responses to NA (\circ) in the rat isolated common carotid artery. Results are expressed as % of the maximum response in the absence of antagonist. Each point represents mean \pm S.E. mean (n = 6-8).

Solutions and drugs

The composition of the modified Krebs-Henseleit solution was as follows (in mM): NaCl 118.4, NaHCO_3 25, KCl 4.7, KH_2PO_4 1.6, MgSO_4 0.6, CaCl_2 2.5 and glucose 11. Na_2EDTA (23 μ M) was also included in the Krebs solution in all experiments to prevent degradative oxidation of NA and propranolol (1 μ M) and cocaine hydrochloride (10 μ M) were also included to inhibit β -adrenoceptors and neuronal uptake of NA, respectively. The following compounds were used: prazosin HCl (Pfizer); rauwolscine (Roth); UK-14304 (Pfizer); (-) - phenylephrine HCl (Sigma); (-) - noradrenaline bitartrate (Sigma); propranolol HCl (Sigma); cocaine HCl (MacCarthys). All drugs were dissolved in distilled water. All concentrations of the drugs used are expressed as final concentration in the organ bath.

RESULTS

The mixed α -adrenoceptor agonist noradrenaline (non-

selective), phenylephrine (selective α_1) and UK-14304 (selective α_2) produced concentration-dependent contractions in the rat isolated common carotid artery (Fig. 1c). The rank order of potencies for these agonists were as follows: NA > PE > UK-14304. Consecutive CCRC's to noradrenaline, phenylephrine and UK-14304 were reproducible, and there was a small but non-significant change in the maximum response with time.

Effects of α_1 -adrenoceptor agonists and antagonists

Noradrenaline produced isometric contractions with a pD_2 of 7.97 and a maximum contraction of 1.13 ± 0.03 g. Phenylephrine produced isometric contractions with a pD_2 of 7.07 and a maximum contraction of 0.96 ± 0.02 g. The maximum responses to noradrenaline and phenylephrine were not significantly different, but to UK-14304 was smaller than for the other two agonists. Relative to noradrenaline its intrinsic activity was 0.37. UK-14304 did not clearly produce maximum (Fig. 1c). Responses to these agonists were analyzed using antagonists proposed to be selective as follows: prazosin, se-

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lective for α_1 c.f. α_2 . Prazosin produced concentration dependent parallel rightward displacement of noradrenaline and phenylephrine CCRC (Fig. 1a,b). The pA_2 value for prazosin versus noradrenaline was 10.05 and the slope of Schild plot was not significantly different from one (1.045), indicating competitive antagonism (Table I). The pA_2 value for prazosin versus phenylephrine was 9.81 and the slope of Schild plot was very near to unity (0.98), indicating competitive antagonism.

UK-14304 produced isometric contraction with a pD_2 of 5.1 and maximum contraction of $0.44 \pm 0.01g$. Rauwolscine produced a small rightward displacement of the UK-14304 CCRC. Lack of a true maximum contraction prohibits a pA_2 value for rauwolscine versus UK-14304, but the small sensitivity of rauwolscine and high sensitivity to prazosin strongly implicates α_1 agonism. Rauwolscine produced a small rightward displacement of the noradrenaline CCRC, showing the small effect of rauwolscine (Fig. 1d), indicating that the contractions of noradrenaline are not mediated by α_2 -adrenoceptors. The high sensitivity of noradrenaline to prazosin strongly implicates α_1 agonism.

DISCUSSION

This study demonstrates that in the rat common carotid artery the dominant population of postjunctional α -adrenoceptors mediating contraction of smooth muscle to adrenoceptor agonists is α_1 . It has long been known that phenylephrine is a relatively selective α_1 -adrenoceptor agonist c.f. α_2 and that prazosin is a highly selective α_1 -adrenoceptor antagonist with a more than 250 times higher affinity for the α_1 -adrenoceptor than for the α_2 -adrenoceptor.¹² In this study contractions to noradrenaline and phenylephrine were relatively sensitive to low concentrations of prazosin ($\ln M$). Responses to noradrenaline were insensitive to the selective α_2 antagonist rauwolscine. In high concentration rauwolscine ($1\mu M$) caused only a small shift to the right of the noradrenaline CCRC. UK-14304 is a selective α_2 -adrenoceptor agonist in a variety of preparations.¹⁰ UK-14304 produced a concentration-dependent contraction with a maximum $41.3 \pm 4.47\%$ of NA ($1\mu M$). In high concentrations rauwolscine ($1\mu M$) caused only a small shift on responses mediated by UK-14304. These results clearly show the dominance of postjunctional α_1 -adrenoceptors in isolated carotid artery. It has been difficult to demonstrate contraction to postjunctional α_2 -adrenoceptors in isolated large arteries.^{7,13} Nevertheless, there are a few old reports on the presence of α_1 - and α_2 -adrenoceptors in arterial preparations and α_2 -adrenoceptors in the veins.¹⁴ The adrenergic system normally functions in the vasculature predominantly through local activation of α_1 -adrenoceptors, whereas there is some pharmacological evidence for existence of post-junctional α_2 -adrenoceptors that mediate vascular contraction.^{5,15} Our finding of α_1 -adrenoceptors in this large artery is consistent

with most of the intervening literature that in the adrenergic system contractions of blood vessels are usually mediated through local activation of α_1 -adrenoceptors.^{7,12,16,17} These findings are against the existence of functional α_2 -adrenoceptors in the cephalic artery, and therefore against their postulated involvement in contraction or modulation of the adrenergic system in this artery.

REFERENCES

1. Oliver G, Schafer EA: The physiological effects of extracts from suprarenal capsules. *J Physiol* 18: 230-237, 1985.
2. Elliot TR: The action of adrenaline. *J Physiol (London)* 32: 401-467, 1905.
3. Ahlquist RP: A study of the adrenotropic receptors. *Am J Physiol* 153: 586-600, 1948.
4. Langer SZ: Presynaptic regulation of catecholamine release. *Biochem Pharmacol* 23: 1793-1800, 1974.
5. Docherty JR, McGrath JC: A comparison of pre- and post-junctional potencies of several alpha-adrenoceptor agonists in the cardiovascular system and anococcygeus muscle of the rat. Evidence for two types of post-junctional α -adrenoceptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 312: 107-116, 1980.
6. Starke K, Endo T, Taube HD: Relative pre- and postsynaptic potencies of α -adrenoceptor agonists in the rabbit pulmonary artery. *Naunyn-Schmiedeberg's Arch Pharmacol* 291: 55-78, 1975.
7. McGrath JC: Evidence for more than one type of postjunctional α -adrenoceptor. *Biochem Pharmacol* 31: 467-484, 1982.
8. Weitzell R, Tanaka T, Starke K: Pre- and postsynaptic effect of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. *Naunyn-Schmiedeberg's Arch Pharmacol* 308: 127-136, 1979.
9. Grant JA, Scrutton MC: Interaction of selective alpha-adrenoceptor agonists and antagonists with human and rabbit blood platelets. *Br J Pharmacol* 71: 121P, 1980.
10. Cambridge D: UK-14304, a potent and selective α_2 -agonist for the characterisation of α -adrenoceptor subtypes. *Eur J Pharmacol* 72: 413-415, 1981.
11. Ruffolo RR, Nichols AJ, Stadel JM, Hieble JP: Pharmacologic and therapeutic applications of α_2 -adrenoceptor subtypes. *Ann Rev Pharmacol Toxicol* 32: 243-279, 1993.
12. McGrath JC, Daly CJ: Viewing adrenoceptors: past, present and future; commentary and a new technique. *Pharmacol Commun* 6: 269-279, 1995.
13. Nase GP, Boegehold MA: Postjunctional α_2 -adrenoceptors are not present in proximal arteries of rat intestine. *Am J Physiol* 274 (Heart Circ Physiol 43): H202-H208, 1998.
14. Kobinger W, Pichler L: α_1/α_2 Selectivity ratio in a series of agonists and their relation to pre/postsynaptic activity ratios. *Eur J Pharmacol* 91: 129-133, 1983.
15. Callow ID, Campisi P, Lambert MI, Feng Q, Arnold MO: Enhanced in vivo α_1 - and α_2 -adrenoceptor-mediated vasoconstriction with indomethacin in humans. *Am J Physiol* 275 (Heart Circ Physiol 44): H837-H843, 1998.
16. Blue DR, Criag DA, Ransom JT, Camacho PA, Clarke DE: In vivo studies on alpha-adrenergic receptor subtypes in human veins. *Naunyn-Schmiedeberg's Arch Pharmacol* 344: 302-307, 1991.

17. Bockman CS, Gonzalez-Cabrera I, Abel PW: Alpha-2 adrenoceptor subtype causing nitric oxide-mediated vas-

cular relaxation in rats. *J Pharmacol Exp Ther* 278: 1235-1243, 1996.

