

## Basic Sciences

# POTENTIATION OF RESPONSES TO UK-14304 IN RAT ISOLATED COMMON CAROTID ARTERY BY ANGIOTENSIN

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### ABSTRACT

The selective  $\alpha_2$ -adrenoceptor agonist UK-14304 produces a small vasoconstrictor response in the rat isolated carotid artery. The purpose of the work presented here was to investigate whether stimuli that produce submaximal contraction would potentiate responses to UK-14304. Male Wistar rats were killed by overdose with pentobarbitone sodium, after which the left and right common carotid arteries were removed. The 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% O<sub>2</sub> plus 5% CO<sub>2</sub>. The preparations were allowed to equilibrate for an hour. Cumulative concentration-response curves (CCRC) were constructed in a cumulative manner by increasing the concentration of the agonists in half-log increments. When antagonists were used, the preparations were incubated at least for 45 minutes with the drugs prior to the onset of a second CCRC. Angiotensin II (AII) and other contracting factors were added approximately 10-15 min prior to the onset of CCRC to an agonist. After inducing tone with low concentrations of the thromboxane A<sub>2</sub> mimetic agent U-46619 (1nM), 5HT (0.5-1  $\mu$ M) and phenylephrine (10-nM), exposure of the preparation to UK-14304 resulted in concentration dependent contractions to this agonist. The sensitivity and maximum response of the preparation to UK-14304 were not changed. Inducing tone with AII (0.01  $\mu$ M) produced a significant leftward shift in the CCRC to UK-14304 ( $p < 0.05$ ). Thus submaximal contraction with AII (0.01  $\mu$ M) increased responses significantly, but inducing tone with phenylephrine, U-46619 and 5HT had no effect on responses to UK-14304. The  $\alpha$ -adrenoceptor antagonists prazosin and rauwolscine were examined to see whether UK-14304's main action in the presence of AII remained via  $\alpha_1$ . The potentiated responses were prazosin sensitive and rauwolscine resistant, indicating an increasing effect mediated by  $\alpha_1$ -adrenoceptors.

*MJIRI, Vol. 15, No. 2, 83-88, 2001.*

**Keywords:**  $\alpha$ -adrenoceptors, UK-14304, prazosin, rauwolscine, angiotensin II

## INTRODUCTION

Oliver & Schafer<sup>1</sup> demonstrated that injection of adrenal gland extracts caused a rise in arterial pressure *in vivo*. The initial view about adrenoceptors held by Elliot<sup>2</sup> was that adrenaline was the most likely mediator of sympathetic neurotransmission. The  $\alpha$ -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 Ahlquist<sup>3</sup> concluded that the differences in potency orders could only be explained by assuming differences in the receptors. Subsequent work in this field, using a number of selective agonist and antagonist drugs, has confirmed the existence of  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors postsynaptically.<sup>4</sup> In general,  $\alpha$ -adrenoceptors of the  $\alpha_1$  type are most effectively activated by phenylephrine and antagonised by prazosin, selective for the  $\alpha_1$ -adrenoceptors.<sup>5</sup> Responses to  $\alpha$ -adrenoceptor agonists in the vast majority of isolated vascular preparations, particularly arterial vessels, have been shown to be sensitive to prazosin.<sup>6</sup> The rauwolfia alkaloids rauwolscine and yohimbine were originally shown to be highly selective  $\alpha_2$ -antagonists.<sup>7</sup> UK-14304 is a full agonist at  $\alpha_2$ -adrenoceptors in various pharmacological preparations.<sup>8</sup> The action of  $\alpha_2$ -adrenoceptor agonists may be dependent on the type of other vasoconstrictor agents and it has been suggested that stimulation with contractile agents may potentiate  $\alpha_2$ -adrenoceptor responses.<sup>9</sup> This could have functional significance, as noradrenaline stimulation causes changes in most vascular resistance via  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors.<sup>10</sup> In the rat isolated carotid artery, UK-14304—a selective  $\alpha_2$ -adrenoceptor agonist—produced contractions in high concentrations but responses were small as reported in most large vessels in different species.<sup>11</sup> The present study was designed to investigate whether low concentrations of different contracting factors could modify the small constrictor response obtained by UK-14304 in this artery. We also determined whether the modulating effect of AII on vascular responsiveness of UK-14304 depends on activation of  $\alpha_1$ - or  $\alpha_2$ -adrenoceptors.

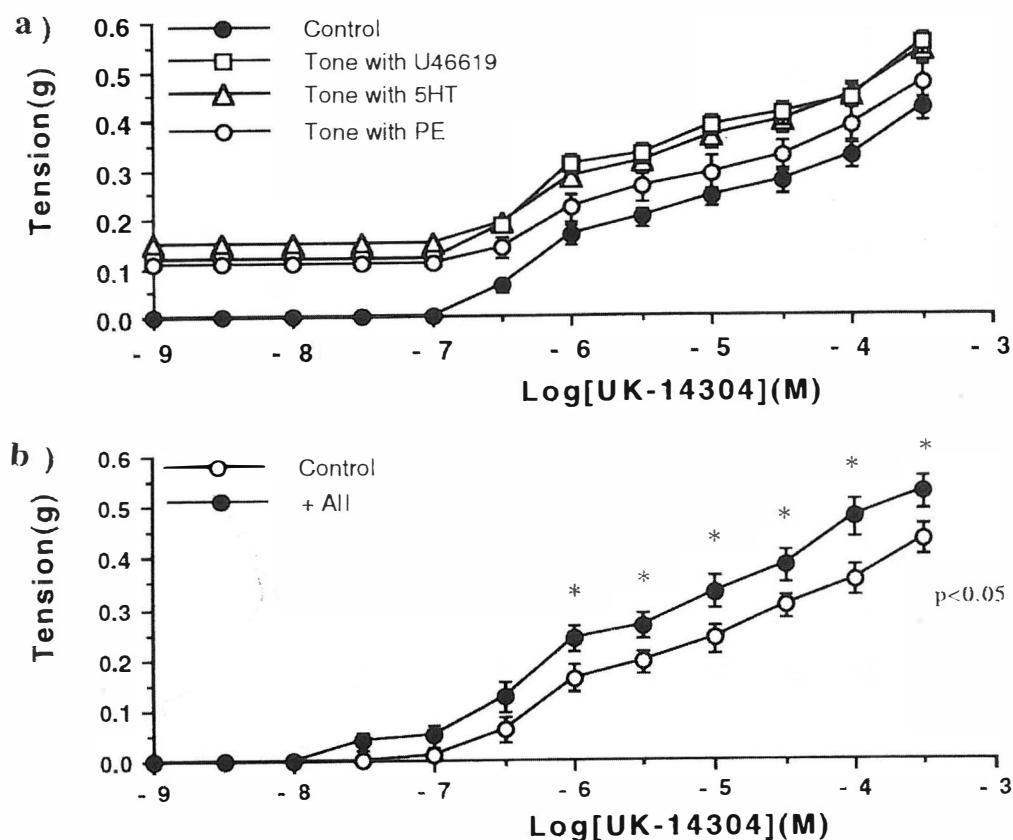
## MATERIAL AND METHODS

Common carotid arteries (700  $\mu$ m in lumen diameter) were obtained from male Wistar rats, weighing 320-400g, which were killed by overdose with pentobarbitone sodium (i.p. injection). A pair of common carotid arteries were easily dissected out and placed in cold, oxygenated modified Krebs-Henselite solution (Krebs). The arteries were cleaned of any extraneous connective tis-

sue using fine scissors. Each preparation was cut transversely in to 3-4mm rings and suspended between thick wire supports. 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%O<sub>2</sub> plus 5% CO<sub>2</sub>.<sup>12</sup> The rings were then placed under resting tension at 2.5-3g for each group of arterial rings of 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 noradrenaline (1 $\mu$ 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 competitive antagonists such as 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. AII and other contracting factors with low concentrations was added approximately 10-15 min prior to the onset of CCRC to an agonist. Results are expressed as mean $\pm$ standard error of mean (s.e.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 ANOVA. A value of  $p < 0.05$  was taken as statistically significant.

## Solutions and drugs

The composition of the modified Krebs-Henselite solution was as follows (in mM): NaCl 118.4, NaHCO<sub>3</sub> 25, KCl 4.7, KH<sub>2</sub>PO<sub>4</sub> 1.6, MgSO<sub>4</sub> 0.6, CaCl<sub>2</sub> 2.5 and glucose 11. Na<sub>2</sub>EDTA (23 $\mu$ M) was also included in the Krebs in all experiments to prevent degradative oxidation of NA, and propranolol (1 $\mu$ M) and cocaine hydrochloride



**Fig. 1.** Effects of inducing tone **a)** with 5HT (0.5-1 $\mu$ M), PE (10 nM) or U-46619 (1nM); **b)** effect of presence of angiotensin AII (10 nM) on responses to UK-14304 in rat isolated common carotid artery. Results are expressed as tension (g).

Each point represents the mean $\pm$ S.E.M (n= 6-8). Statistically significant differences are represented by \* $p$ <0.05, using paired Student's t-test. Value of  $p$  shows comparison of two curves by one way ANOVA.

(10  $\mu$ M) were also included to inhibit  $\beta$ -adrenoceptors and neuronal uptake of NA respectively. The following compounds were used: (-)-noradrenaline bitrate (Sigma); propranolol HCl (Sigma); cocaine HCl (MacCarthys); prazosin HCl (Pfizer); rauwolscine (Roth); UK-14304 (Pfizer); (-)-phenylephrine HCl (Sigma); U-46619 (Upjohn); 5HT (Sigma). All drugs except U-46619 were dissolved in distilled water. U-46619 was initially dissolved in high-performance liquid chromatography-grade absolute ethanol, with subsequent dilutions made in distilled water. All concentrations of the drugs used are expressed as final concentration in the organ bath.

## RESULTS

### Effect of inducing tone with phenylephrine, U-46619,

### 5HT or angiotensin II on responses to UK-14304

A series of experiments were conducted to study whether stimuli that produce submaximal contraction would potentiate responses to UK-14304.

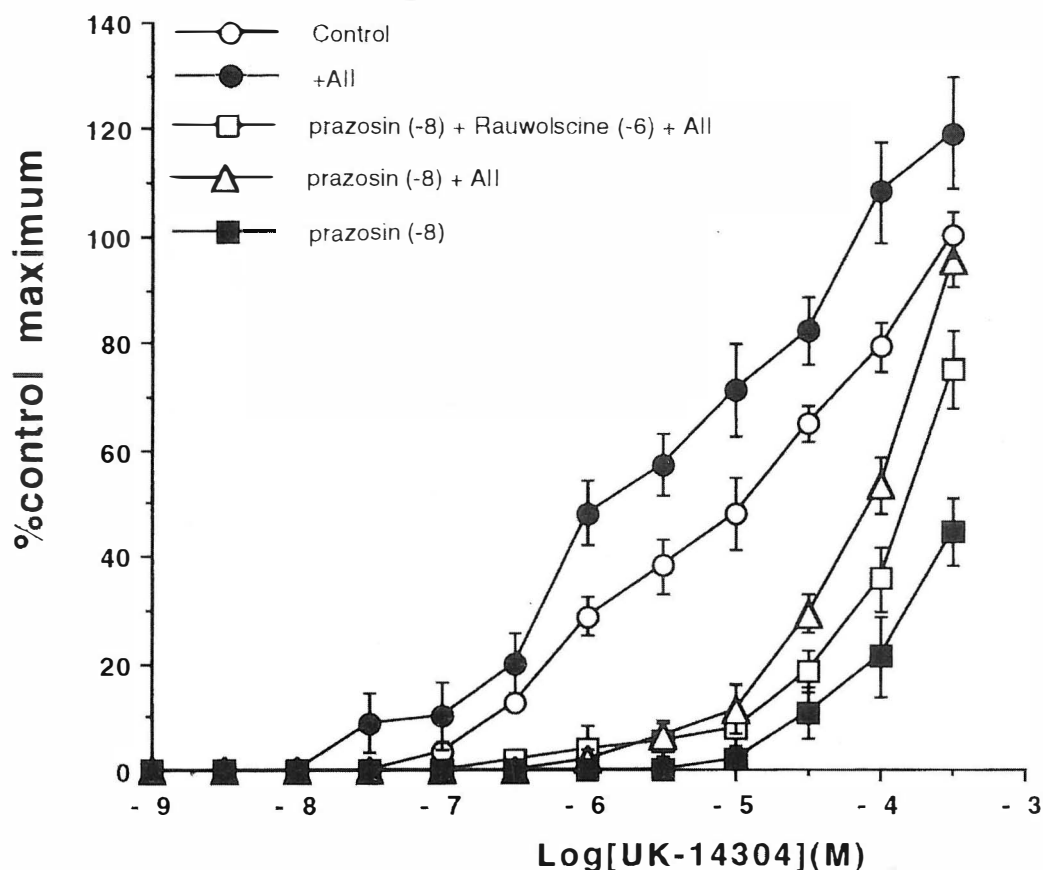
#### 1-Phenylephrine

A low concentration of phenylephrine (10 nM) produced a sustained contraction equivalent to 16.8 $\pm$ 3.3% of the noradrenaline contraction (1 $\mu$ M). After inducing tone, exposure of the preparation to UK-14304 resulted in concentration dependent contractions to this agonist. The sensitivity and maximum response of the preparation to UK-14304 were not changed (Figure 1).

#### 2- U-46619

The thromboxane A<sub>2</sub> mimetic agent U-46619 (9, 11-

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**Fig. 2.** Analysis of  $\alpha$ -adrenoceptor contractile response to UK-14304 after potentiation with AII (10 nM) and in the presence of prazosin or rauwolscine on responses to UK-14304. Responses are expressed as % of the maximum response of the control CCRC to UK-14304 in the absence of antagonist or AII. Concentration is shown as Log (M) in the graphs. Each point represents mean  $\pm$  S.E.M. (n = 6).

dideoxy-11a, 9a-epoxymethanoprostaglandin F $2\alpha$ ) (1nM) produced a sustained contraction, equivalent to 17.19 $\pm$ 6.14% of the noradrenaline contraction (1 $\mu$ M) in the rat isolated carotid artery. In the presence of U-46619, the sensitivity and maximum response of the preparation to UK-14304 were not changed (Figure 1).

### 3- 5-Hydroxytryptamine (5HT)

5HT (0.5-1 $\mu$ M) produced a contraction equivalent to 22.4 $\pm$ 5.4% of noradrenaline's contraction (1 $\mu$ M). Inducing tone with this agent was not associated with a significant enhancement or uncovering of responses to UK-14304 (Figure 1).

### 4- Angiotensin II (AII)

AII (0.01  $\mu$ M) produced a transient contraction, which returned to baseline after 5-10 min, in the rat isolated common carotid artery. This response was equivalent to 97 $\pm$ 3.23% of noradrenaline contraction (1 $\mu$ M) in each preparation. There was a small but significant leftward shift in the CCRC to  $\alpha_2$ -adrenoceptor agonist UK-14304

in the presence of AII ( $p < 0.05$ ) (Figure 1).

### UK-14304 mediated contraction in the presence of AII is $\alpha_1$ -mediated

Since AII could potentiate the contractile effect of UK-14304, the  $\alpha$ -adrenoceptor antagonists prazosin and rauwolscine were examined to see whether UK-14304's main action remained via  $\alpha_1$ .

Figure 2 shows the effects of rauwolscine (1 $\mu$ M) and prazosin (10 nM) on the concentration-response curves to UK-14304 in preparations pretreated with AII. In the presence of prazosin (10 nM) and AII (10 nM), responses to UK-14304 were not significantly different from preparations that had prazosin alone although there was a tendency to increase. By adding rauwolscine (1  $\mu$ M) in tissues that had prazosin (10 nM) and AII (10 nM), the concentration-response curve to UK-14304 had a small shift to the left (Figure 2). These results show that the potentiated responses in the presence of AII (10 nM) were prazosin sensitive and rauwolscine resistant.

## DISCUSSION

As shown in the present study, UK-14304 produced a small vasoconstrictor response in the isolated rat carotid artery. Attempts to potentiate responses to UK-14304 with synergistic agents showed: i) inducing tone with phenylephrine, U-46619 and 5HT had no effect on responses to UK-14304; ii) AII increased responses to UK-14304.

Since the original demonstration of postjunctional  $\alpha_2$ -adrenoceptors in the pithed rat,<sup>6</sup> identification of this subtype in isolated vascular preparations, i.e., responding to noradrenaline and resistant to prazosin or a suitable  $\alpha_1$ -adrenoceptor antagonist, has proved very difficult. It has been reported that activation with a physiological stimulant, namely AII, reveals a quiescent population of  $\alpha_2$ -adrenoceptors in the saphenous artery, although the response was mediated entirely by  $\alpha_1$ -adrenoceptors in the absence of AII. This is associated with a marked increase in the sensitivity of the preparation (up to 300 fold) to the  $\alpha_2$ -adrenoceptor agonist UK-14304 in the presence of AII.<sup>9</sup> Similarly, in the perfused isolated tail of the rat  $\alpha_2$ -adrenoceptor-mediated responses are observed only after raising tone with, for example, vasopressin or endothelin.<sup>13</sup> Similarly, phenylephrine-induced tone has been shown to uncover responses to  $\alpha_2$ -adrenoceptor agonist BHT-920 in the canine portal vein.<sup>14</sup> Furthermore,  $\alpha_2$ -adrenoceptor-mediated responses to BHT-920 in the plantaris artery of the dog are only apparent in the presence of Bay K-8644.<sup>15</sup> In another experiment angiotensin II potentiated sympathetic transmission in rat hind limb circulation.<sup>16</sup>

In the present study activation of isolated common carotid artery with AII (10 nM) caused a small but significant increase in the sensitivity of the preparation to UK-14304 ( $p < 0.05$ ). However, analysis using antagonists showed that responses to UK-14304 after potentiation by angiotensin were prazosin-sensitive, rauwolscine-resistant, and therefore by definition  $\alpha_1$ -adrenoceptor-mediated. This is in contrast to earlier in which inducing tone with phenylephrine or endothelin and presence of AII introduced rauwolscine-sensitive responses to UK-14304 in the isolated vascular bed of the rat tail and saphenous artery.<sup>9,12</sup> The facilitatory action of AII on postjunctional  $\alpha_2$ -adrenoceptor-mediated responses in the saphenous artery under normal circumstances is apparent.<sup>17</sup> In this investigation the potentiating effect of AII on UK-14304-mediated responses in the presence of prazosin was rauwolscine-resistant, consistent with  $\alpha_1$ -adrenoceptor activation. In conclusion, with regard to potentiation by prior exposure to AII it is theoretically possible that UK-14304 exerts its actions through combined  $\alpha_1$  and  $\alpha_2$  activation, but the effectiveness of prazosin and the ineffectiveness of rauwolscine except

in non-selective concentrations makes the simplest explanation that it is working entirely via  $\alpha_1$ .

Inducing tone with phenylephrine, U-46619 or 5HT had no effect on responses to UK-14304. However AII increased responses. The mechanism of increased responses induced by AII is not readily apparent. It is possible that in the presence of contracting agents, an increase in intracellular calcium or sensitivity to intracellular calcium may be enhanced.<sup>18,19</sup> AII-induced potentiation could be due to alterations at the receptor levels or through an interaction at second messengers leading to an increased affinity of UK-14304 for the  $\alpha$ -adrenoceptor. Alternatively the potentiation of the response to UK-14304 is by assuming that angiotensin acts on a common pathway linked to different receptors which is part of the excitation-contraction coupling mechanism.<sup>20,21</sup> The results of the present study demonstrate that AII potentiates the responses to UK-14304 through activation of the  $\alpha_1$ -adrenoceptor. In conclusion, we have demonstrated that an enhanced contraction to UK-14304 in the rat isolated carotid artery can be obtained by prior pharmacological manipulation.

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