Angiotensin tachyphylaxis and the uptake of [^{14}C]angiotensin in guinea-pig aortic strips

The development of tachyphylaxis in response to angiotensin in guinea-pig isolated aortic strips has been attributed to a change in the receptor mechanism(s) for angiotensin (Palaic & LeMorvan, 1971). We now report the correlation of the change in reactivity to angiotensin and the uptake of [^{14}C]angiotensin in aortic strips.

The thoracic aorta from male guinea-pigs, 250–300 g, was cut spirally in a strip approximately 6 mm wide and 3 cm long. This was divided into two equal parts by longitudinal dissection to give two matching strips from one animal. The strips were mounted in 10 ml baths in Krebs solution at 37°, with 10^{-5}M of EDTA and allowed to relax for 2 h. One strip had drugs, while the other served as the control. The isotonic contractions were recorded on a Physiograph (E & M Co.).

Only concentrations of angiotensin giving maximal responses (around 10^{-7}M and more) were capable of producing tachyphylaxis (Palaic & LeMorvan, 1971). Because of the heterogeneity of receptors in aortic strips, it is impossible to estimate the contractile response to one agonist on the basis of the contractile response to another (Altura & Altura, 1970). Therefore we used only successive doses of the same concentration of angiotensin to produce tachyphylaxis.

The degree of tachyphylaxis achieved after successive maximal doses of angiotensin increased with duration of contact between the angiotensin and the aortic preparation and with decreased recovery time after each contraction, but recovery time could not be shortened to less than 10–12 min, that being the minimum time required for the strip to relax to the base line. A 3 min contact with the agonist and 12 min recovery time, was therefore employed throughout. The time required to achieve a 50% decrease from the initial maximal response (DMR50) may serve as a reliable index of tachyphylaxis. DMR50 values for different concentrations of angiotensin were plotted and the straight line obtained showed tachyphylaxis to be directly proportional to the concentration of angiotensin. The theoretical implications that may possibly be considered from such a relation could have a bearing on the change in receptor reactivity with respect to time and the relative number of receptors involved. One could also postulate the existence of "negative" tachyphylaxis for submaximal doses, this in fact being an autostimulation or autopotentiation. The phenomenon has been already described by Godfraind (1968) and we have observed it with submaximal doses of angiotensin in guinea-pig aorta. The nature of this interesting phenomenon is unknown.

Table 1. *Uptake of ^{14}C-angiotensin by guinea-pig aortic strips and vas deferens.* Aortic strips were made tachyphylactic to 10^{-6}M angiotensin and then incubated with 1·6 μg/ml of [^{14}C] angiotensin (125 nCi/ml) at 37° for 15 min. Results expressed as d min^{-1} mg^{-1} of dry tissue; individual results. Statistical analysis was made using the paired *t*-test.

Aorta		Vas deferens
Control	Tachyphylactic	
759	882	185
682	911	216
771	877	202
941	967	185
1090	1188	221
1234	1470	
912 ± 88·0	1049 ± 96·6	202 ± 20·9
	$P < 0{\cdot}01$	

A major, as yet unresolved problem in the pharmacology of smooth muscle is how to correlate the contractile response with the binding capacity of muscle for an agonist. It was thought that a smooth muscle preparation in which a change in reactivity, such as tachyphylaxis, is produced by the agonist itself might be a useful model for such a study. In our experiments shown in Table 1, aortic strips were firstly made tachyphylactic to 10^{-6}M angiotensin. They were then transferred to a beaker containing 1·6 μg/ml of [^{14}C]angiotensin (125 nCi/ml) in 2 ml of Krebs solution, and incubated in a Dubnoff shaker at 37° for 15 min. The strips were subsequently washed and prepared for counting in a Packard liquid scintillation counter. A vas deferens taken from the same animal was incubated and counted in the same fashion as the aortic strips. Since the vas deferens does not respond to angiotensin, it was considered as a smooth muscle without specific receptors for angiotensin. The results are expressed as d min^{-1} mg^{-1} of dry tissue. Statistical analysis was made using the paired *t*-test.

Our results show that the uptake of [^{14}C]angiotensin is significantly higher in tachyphylactic strips than in the controls ($P<0{\cdot}01$). This was not due to the change in extracellular space, since the inulin space was the same in tachyphylactic strips as in the controls. The amount of [^{14}C]angiotensin bound to the vas deferens was only one quarter of that found in aortic strips, indicating the possible degree of non-specific binding to the smooth muscle.

The increased binding of [^{14}C]angiotensin indicates that the number of receptive sites is increased during the development of tachyphylaxis. This might be due to the induction of new receptors, either by *de novo* synthesis or by conformational changes of the existing receptor proteins.

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