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Long-lasting inhibition of angiotensin response in rats by depot administration of octanoyl-[Leu^8]-angiotensin II

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Depot administration of a lipophilic angiotensin II (AII) antagonist was tested for obtaining prolonged inhibition of the pressor response to AII in rats. Intramuscular injections of 1·5 or 5·0 mg of octanoyl-[Leu^8]AII (oct-LAII), in oil solution produced the same degree of AII inhibition either 6 h or 24 h after the injection. The inhibition was comparable to that expected from the continuous intravenous infusion of oct-LAII at the rate of 1·2 μg kg^{-1} min^{-1}. The prolonged effect of intramuscular injections of oct-LAII in oil solution may be useful for chronic studies of physiopathological states involving the renin-angiotensin-aldosterone system.

The study of the possible role played by the renin-angiotensin-aldosterone axis in human hypertension (Case et al 1977) and in other pathological situations (Flamenblaum 1973) has been stimulated by the availability of inhibitors of the renal pressor system (Brunner et al 1976; Needleman et al 1976; Postemak et al 1977). Four types of inhibitor are available: blockers of renin secretion, renin inhibitors, converting enzyme antagonists and competitive inhibitors of angiotensin II (AII). Among the numerous compounds of the latter type that have been described, [1-sarcosine, 8-alanine]angiotensin II (Saralasin) (Pals et al 1971) has been the most widely employed. This compound, however, shares with all the other available angiotensin antagonists the inconvenience of a short duration of action, necessitating intravenous infusion for the maintenance of the inhibition. Attempts to design longer-lasting angiotensin antagonists by increasing their resistance to enzymic degradation have not been successful (Turk et al 1976), and the use of depot administration (Khosla et al 1977) is hampered by the high solubility in water and low lipophilic character of these compounds.

The recent synthesis of octanoyl-[8-leucine]-angiotensin II (oct-LAII), a lipophilic angiotensin antagonist (Paiva et al 1977), allowed us to investigate the effect of depot administration of this compound on the pressor response of rats to AII, as a preliminary step to its use for chronic inhibition studies.

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MATERIALS AND METHODS

Oct-LAII was synthesized by the solid phase method (Paiva et al 1977). It was shown to be homogeneous by paper electrophoresis in three buffers (pH 2·8, 4·9 and 9·9) and by thin layer chromatography on silica gel with three different solvent systems (Paiva et al 1977). The pure peptide was dissolved in sterilized corn oil just before use. AII was synthetic [1-asparagine, 5-valine]-angiotensin II (Ciba), dissolved (0·4 mg litre^{-1}) in a sterilized 20% ethanol solution.

Male Wistar rats, 190–269 g, were anaesthetized by intraperitoneal injection of sodium pentobarbitone (50 mg kg^{-1}) and placed on a heated table at 38° C. The carotoid artery and jugular vein of the same side were cannulated. The arterial catheter was connected to a pressure transducer for continuous recording of the average blood pressure with a Beckman type RP Dynograph recorder. The jugular cannula was used for intravenous administration of drugs according to the following protocol. 1) 2·5 ng AII (0·005 ml) was injected and if the pressor response was less than 6 mm Hg the rat was discarded as unresponsive; 2) pentolinium tartrate (17 mg kg^{-1}) was injected to increase AII sensitivity and stabilize the basal blood pressure; 3) after 10–15 min, a dose-response curve to AII was obtained by measuring the averages of the pressor responses to three doses (2·5, 1·25 and 5·0 ng, in that order) administered 2–4 times each, at times that allowed full recovery from each dose before the next injection. The carotid and jugular vein were then ligated, and the skin was sutured.

The animals were then separated into three groups: (a) a control group (12 rats) received an intramuscular injection of 1 ml corn oil in the lower lumbar region; (b) 12 rats received a similar intramuscular injection of 1·5 mg oct-LAII in 1 ml corn oil; (c) 13 rats received 5·0 mg oct-LAII in 1 ml corn oil intramuscularly.

After recovering from anaesthesia the animals were placed in metabolic cages with free access to food and water. Six hours later, 6 rats from each group were anaesthetized with sodium pentobarbitone and the remaining carotid artery and jugular vein were cannulated as before. Steps 2 and 3 of the above described protocol were then performed, with the difference that a smaller dose of pentolinium tartrate was used (6 mg kg^{-1}) to avoid cumulative action of that drug. The remaining rats of the three groups were submitted to the same procedure 24 h after the oct-LAII or oil injections. In the groups treated with 5 mg oct-LAII, the responses to AII doses higher than 5·0 ng (up to 35 ng) were also tested, in order to extend the dose-response curve to the same level of response observed before the treatment. The AII responses before and after treatment with oct-LAII or oil, in all groups, were analysed according to a linear model whose parameters were estimated by the least squares method. Multiple comparisons were made by testing linear hypotheses and $P = 0{\cdot}01$ was taken as the significance level. Results are presented as means with one standard deviation.

RESULTS

Ligature of carotid arteries and jugular veins, as well as the intramuscular injection of oil or oct-LAII in oil, did not affect body weight or control blood pressure measured before pentolinium and angiotensin administration. The greatest variations of these parameters occurred in the control group studied 24 h after receiving 1 ml oil, for whom body weight decreased from 186 ± 22 g to 176 ± 27 g and blood pressure increased from 126 ± 21 mm Hg to 145 ± 27 mm Hg. However these variations were non-significant even at a level of $P < 0{\cdot}05$. It has been previously shown that intravenous oct-LAII has a very low pressor activity (0·10 ± 0·02% of AII's activity) with a half life of less than 1 min (Paiva et al 1977).

After pentolinium, blood pressure was reduced by approximately 30% and remained stable throughout the experiment, in all the rats.

The response to AII of the control group was not significantly altered (Table 1), indicating that the results in the other groups were not affected by the ligation of the carotid arteries and jugular veins. On the other hand, in all the groups treated with oct-LAII the AII log dose-response curves were significantly shifted to the right, as illustrated in the example shown in Fig. 1. The ratios between the AII doses that produced the same pressor effect before and after treatment with 5 mg of the inhibitor were 4·0 ± 1·5 (6 h) and 4·8 ± 1·0 (24 h). The data obtained using the three chosen AII test doses (1·25, 2·50 and 5·00 ng) are given in Table 1.

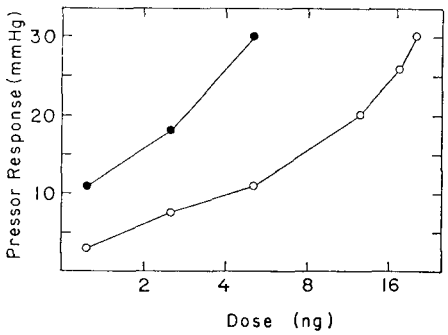

FIG. 1. Representative AII log-dose responses curves obtained in one rat before (●) and 6 h after (■) intramuscular injection of 5 mg of oct-LAII in corn oil. Ordinate: pressor response (mm Hg). Abscissa: AII dose (ng).

Table I. Pressor response (in mm Hg) of the rat to three doses of AII injected intravenously before and after intramuscular injection of oct-LAII in oil (with s.d.).

Treatment	No. of rats	AII = 1·25 ng Before	After	*P*	AII = 2·50 ng Before	After	*P*	AII = 5·00 ng Before	After	*P*
Control, 6 h	6	11 (5)	13 (4)	N.S.	18 (4)	19 (5)	N.S.	27 (7)	28 (5)	N.S.
Control, 24 h	6	9 (3)	7 (3)	N.S.	14 (4)	13 (5)	N.S.	24 (7)	22 (7)	N.S.
1·5 mg oct-LAII, 6 h	6	10 (2)	5 (1)	<0·01	17 (5)	8 (2)	<0·01	27 (7)	15 (4)	<0·01
5 mg oct-LAII, 6 h	6	8 (3)	3 (2)	<0·01	13 (4)	6 (2)	<0·01	25 (5)	10 (2)	<0·01
1·5 mg oct-LAII, 24 h	6	9 (3)	5 (2)	<0·01	15 (3)	10 (1)	<0·01	25 (5)	17 (3)	<0·01
5·0 mg oct-LAII, 24 h	7	8 (3)	4 (2)	<0·01	14 (4)	7 (3)	<0·01	25 (6)	10 (5)	<0·01

The decreases in the pressor responses to all three doses were highly significant in the animals treated with oct-LAII. However, when the AII responses of all the rats treated with 1·5 mg of oct-LAII (both for 6 and 24 h) were compared with those of all rats treated with 5·0 mg of inhibitor, the overall difference was not significant, although for the highest AII dose (5 ng) the inhibition was greater in the animals treated with the larger oct-LAII dose.

No significant differences (overall and for each AII dose) were found between the responses of all rats tested 6 h and those tested 24 h after injection. No difference was found between the responses of the groups treated for 6 and 24 h with either of the two doses of oct-LAII.

DISCUSSION

The responses to AII in the two phases of our experiments were not affected by the ligation of the carotid artery and jugular veins, done after the first dose-response curves had been obtained. AII sensitivity is not affected by pentobarbitone anaesthesia and treatment with pentolinium sensitizes the animals to AII and stabilizes their responses (Smeby & Bumpus 1968). Under these controlled conditions, oct-LAII proved to be a potent AII inhibitor when injected intramuscularly in oil solution. Since no difference was found between the effects of 1·5 or 5·0 mg of the inhibitor, it is possible that still lower doses may be as efficient. Also, the lack of difference between the effects observed at 6 and at 24 h, suggests that a stable plasma concentration of the inhibitor was maintained during the 24 h. This contrasts with the short half life (12 min) of the antagonistic action of oct-LAII when administered intravenously (Paiva et al 1977).

AII inhibition by intramuscular injection of oct-LAII in oil was evidenced by a dose-ratio around 4·0, which is similar to that attained by intravenous infusion at 1·2 $\mu g\,kg^{-1}\,min^{-1}$ (Paiva et al 1977). The effect of a single intramuscular injection was thus approximately equivalent to that of a total of 0·3–0·4 mg given to an animal of 200 g weight by intravenous infusion for 24 h.

The possibility of obtaining regular and sustained AII inhibition by repeated intramuscular injections of oct-LAII in oil solution may be valuable for chronic studies of the involvement of the renin-angiotensin-aldosterone system in physiopathological states. Such studies in one of our laboratories have already yielded encouraging preliminary results in rats with glycerol-induced acute tubular necrosis and with chronic renal hypertension.

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