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Converting enzyme inhibition in the rat by captopril is accompanied by potentiation of carrageenin-induced inflammation

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1 Hind paw oedema in rats, measured by plethysmography or extravasation of Evans Blue dye into the skin, after subplantar injection of submaximal doses of carrageenin (1–100 μg) was significantly increased for 4 h during kininase II inhibition with captopril (1 mg kg^{-1}, s.c.).

2 Submaximal oedema, as assessed by paw swelling, after subplantar bradykinin (0.1–1.0 μg) was also significantly increased after subcutaneous administration of this dose of captopril, whereas that in response to either histamine (2–20 μg) or prostaglandin E_2 (2 μg) was unchanged.

3 The pain threshold of the paw, injected with carrageenin (1 μg) was lowered significantly after subcutaneous administration of captopril (1 mg kg^{-1}).

4 Potentiation by captopril (1 mg kg^{-1}, s.c.) of paw swelling in response to intraplantar carrageenin (100 μg) or bradykinin (1 μg) was reduced by prior subcutaneous administration of indomethacin (5 mg kg^{-1}).

5 It is suggested that normally, tissue kininase II activity is sufficient to decrease the inflammatory response of the hind paw to carrageenin or bradykinin. After inhibition of kininase II with captopril, bradykinin levels are increased and interact with concomitantly released prostaglandins to potentiate inflammation.

Introduction

Bradykinin is an established mediator of the inflammatory response of tissues to irritant stimuli (Lewis, 1970). Its enzymatic degradation can be brought about by a number of peptidases including the dipeptidyl carboxypeptidase kininase II (Bakhle, 1980). The latter is also the converting enzyme which hydrolyses angiotensin I to angiotensin II (Erdos, 1977). Captopril has been shown to be a specific competitive inhibitor of this enzyme (Ondetti *et al.*, 1977). This therefore suggested the possibility that the drug could be used to investigate the role of kininase II in experimentally induced inflammation. Moreover, block of kininase II during therapy with captopril, by increasing bradykinin levels, could be responsible for the relatively high incidence of skin rash which occurs (Anon, 1980; Luderer *et al.*, 1982). During inflammatory responses of the skin in man, bradykinin has been shown to be released (Michel *et al.*, 1970). For these reasons it was thought to be of interest to determine whether captopril was capable of potentiating experimentally induced inflammation in the laboratory animal.

Methods

Groups of male Wistar rats (100–200 g) were used. Each animal was injected subcutaneously 30 min before the start of the experiment with either captopril (1 mg kg^{-1}) or an equivalent volume of 0.9% w/v NaCl solution (saline) as controls.

Blockade of kininase II by captopril

An estimate of the degree of blockade of kininase II during the period of each experiment was made by examining responses of the arterial blood pressure to intravenous angiotensin I and bradykinin of animals previously given subcutaneously 1 mg kg^{-1} captopril and comparing them to those in controls given saline by the same route. Groups of 5 rats were used. In each, anaesthesia was induced with 4% halothane in oxygen and maintained with intravenous chloralose (80 mg kg^{-1}) after cannulation of the right jugular vein. This was supplemented when necessary with further chloralose (10 mg kg^{-1}, i.v.). The trachea was cannulated. Arterial blood pressure was recorded from the left carotid artery using a Statham pressure

transducer (Gould P23) and displayed on a Grass polygraph (Model 7D). Rectal temperature was maintained at 37°C. Approximately 30 min after the subcutaneous administration of captopril or saline, two logarithmic series of doses of angiotensin I (0.01–1.0 μg) and bradykinin (0.01–30.0 μg), given randomly, were injected intravenously each dose being washed in with 0.25 ml saline. These were subsequently repeated at approximately 30 min intervals for the following 4 h. The degree of inhibition of angiotension I or potentiation of bradykinin, as indicated by the dose-response curves obtained from the control and captopril injected groups, allowed an approximate estimate to be made of the degree of kininase II inhibition in the circulation of the captopril-treated animals.

Hind paw oedema

Oedema was induced in the right hind paws of further groups by subplantar injection (0.1 ml) of submaximal doses of carrageenin (10–1,000 μg ml^{-1}), bradykinin (1–10 g ml^{-1}), histamine (20–200 μg ml^{-1}), or prostaglandin E_2 (20 μg ml^{-1}). The contralateral hind paw received by subplantar injection an identical amount of saline. The volume of each paw was measured by plethysmography every 30 min for the following 4.5 h (Winter *et al.*, 1962). No significant changed occurred in the volume of the left paws injected with saline in either control or captopril-treated groups ($P > 0.05$). The magnitude of the oedema developing in the right paw was therefore assessed as the percentage increase in mean paw volume when compared with that recorded initially (1.1 ml, s.e. mean 0.1). In some groups the effect of indomethacin (5 mg kg^{-1}, s.c.), given 30 min before subcutaneous captopril or saline, was also examined.

Increased vascular permeability in further groups was assessed after subplantar injection of 0.1 ml carrageenin (100 μg ml^{-1}) into the right hind foot and saline into the left, from the extravasation of dye (Harada, *et al.*, 1971) after intravenous injection of Evans Blue dye (80 mg kg^{-1}). Each animal was killed by cervical dislocation, the skin of each hind foot up to the calcaneum removed, the dye extracted and determined colourimetrically.

The effect of captopril on the pain threshold of the inflamed hind foot was examined in other groups. Pain thresholds were determined by the method of Randall & Selitto (1957). After receiving subcutaneously either captopril (1 mg kg^{-1}) or saline as controls, a subplantar injection of carrageenin (0.1 ml, 10 μg ml^{-1}) or saline was given 30 min later into the right foot. The pain threshold was measured 30 min later and then every hour for 4.0 h. It was assessed as that pressure applied to the hind paw by an analgesiometer (Ugo-Basile, Milan) which caused each animal to squeak or struggle.

Statistical analysis was carried out using Student's non-paired *t* test and a 2 way Anova for overall comparison of curves.

All doses are expressed in terms of the salt used. The following drugs were used: captopril (E.R. Squibb & Sons Pty. Ltd), bradykinin triacetate (Sigma), prostaglandin E_2 (Upjohn Co.), histamine dihydrochloride (Calbiochem), indomethacin (Sigma), carrageenin potassium (Sigma), Evans Blue (Searle).

Results

Blockade of kininase II by captopril

Dose-dependent rises and falls in arterial blood pressure followed intravenous injection into anaesthetized rats of angiotensin I and bradykinin respectively. When the mean increase in diastolic pressure was plotted against log dose of angiotensin I, the curve obtained using the animals given captopril approximately 30 min before was found to be shifted significantly ($P < 0.05$) approximately 30 fold to the right of that obtained in the controls. For each group of animals, dose-response curves obtained at approximately 30 min intervals for the following 4 h were not significantly different in position from that obtained initially ($P > 0.05$). All curves did not differ significantly from parallelism. A plot of the mean decrease in diastolic blood pressure against the log dose of bradykinin given approximately 30 min after administration of subcutaneous captopril revealed that the curve was shifted to the left of that obtained from the controls, the depressor effect of bradykinin being potentiated approximately 30 fold ($P < 0.05$). Curves obtained from the captopril-treated group for the following 4 h did not differ significantly in position from that obtained initially. Dose-response curves obtained for bradykinin from controls over the 4 h period were not significantly changed and all curves whether obtained using the controls or the captopril-treated animals did not differ significantly from parallelism.

Oedema

Subplantar injections of 1, 10 or 100 μg carrageenin into groups of 5 rats were followed during the next 4 h by a dose-related increase in paw volume. For each dose of carrageenin the group previously injected with captopril responded with significantly increased swelling when compared with the appropriate control group ($n = 5$) ($P < 0.05$). Figure 1 summarises the data obtained using 100 μg carrageenin. The magnitude of the swelling in the group which received

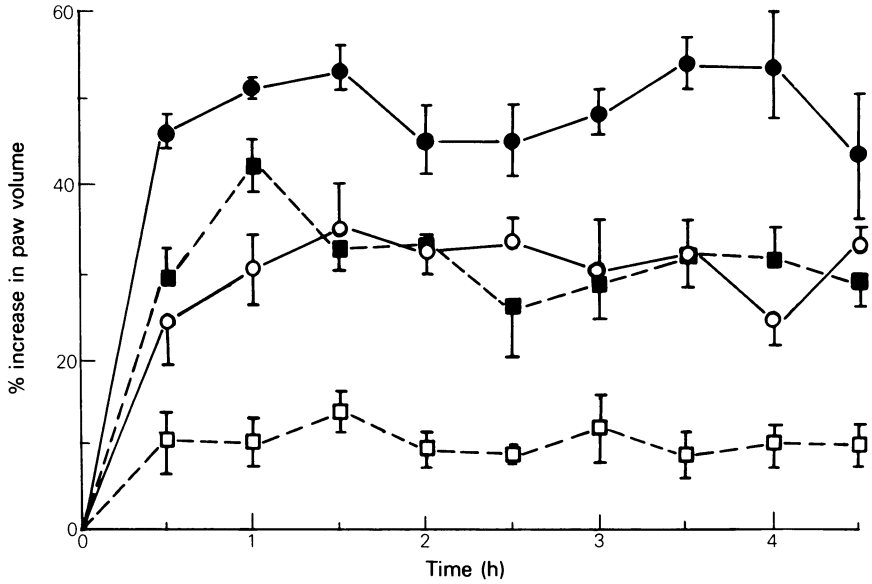

Figure 1 Percentage increase in mean volume of the right paw at intervals after intraplantar injection of carrageenin. Controls (○); captopril 1 mg kg^{-1} s.c. (●); captopril 1 mg kg^{-1} s.c. plus indomethacin 5 mg kg^{-1} s.c. (■); indomethacin 5 mg kg^{-1} s.c. (□). Vertical bars indicate s.e.means (for each group $n = 5$).

captopril was significantly greater than in controls ($P < 0.05$) for at least 4 h. The carrageenin-induced swelling during this time in the group which received indomethacin plus captopril was significantly less than that in the group receiving only captopril ($P < 0.05$). The group which previously received indomethacin alone showed significantly decreased paw swelling when compared with controls ($P < 0.05$).

Intraplantar injection of bradykinin (0.1 μg) into a group of 5 rats was followed by a small increase in mean paw volume (5 ± 2%) 30 min later. In another group ($n = 5$) previously given captopril 1 mg kg^{-1} subcutaneously, this dose of bradykinin caused, at this time, significantly increased swelling (mean 13 ± 1%). This difference between the two groups persisted for 4 h ($P < 0.05$). Figure 2 shows that analogous data were obtained in further groups after giving 1 μg bradykinin by intraplantar injection. The group previously injected with captopril showed significantly increased swelling of the hind paw during the following 4 h, when compared with the hind paw swelling of controls. Also shown are data which indicate that during this time a group which had received indomethacin, in addition to captopril, responded with significantly smaller swelling than the group receiving captopril alone ($P < 0.05$). The magnitude of the swelling after intraplantar bradykinin in the group treated with indomethacin alone was however not significantly different from that of the controls ($P > 0.05$).

In further groups ($n = 5$) intraplantar histamine (2 or 20 μg) or PGE_2 (2 μg) increased mean paw volume (maximum 10 ± 25%, 40 ± 3% and 16 ± 1% respectively), during the following 4.5 h. No significant differences were found during this time between the mean swelling occuring in these groups and that which occurred in corresponding groups injected with captopril.

Figure 3 shows that 1–3 h after intraplantar injection of carrageenin (10 μg) and intravenous Evans Blue, the mean dye content of the skin of the right hind feet of rats previously given captopril was significantly greater than the mean dye content of the controls ($P < 0.05$). The dye contents of the left feet previously injected with saline in both control and captopril groups were below the level of detection (0.5 μg g^{-1}).

A significant decrease ($P < 0.05$) in mean pain threshold of the paw followed 1–4.5 h after intraplantar injection of carrageenin (1 μg) when compared to that of controls in which the paw was injected with saline (Figure 4). The figure also shows that in the group which had received captopril the mean pain thresholds during this time were further reduced ($P < 0.05$).

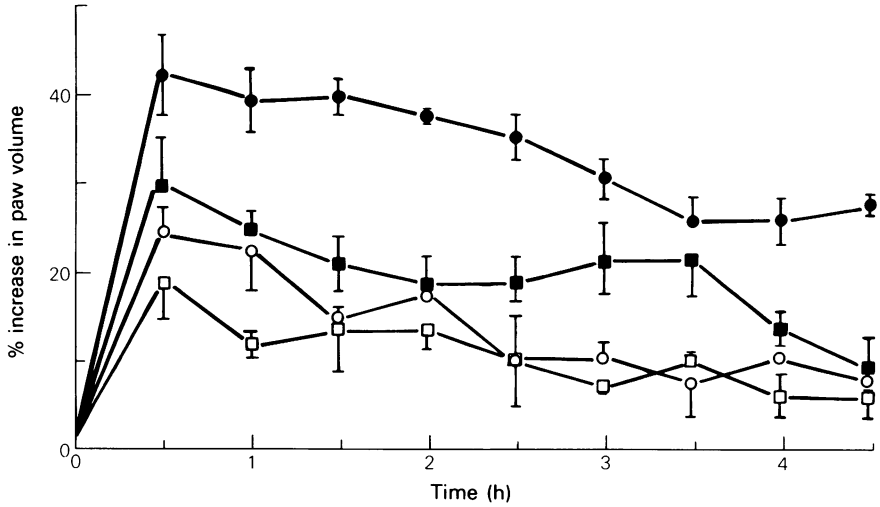

Figure 2 Percentage increase in mean volume of the right paw at intervals after intraplantar injection of bradykinin. Controls (○); captopril 1 mg kg^{-1} s.c. (●); captopril 1 mg kg^{-1} s.c. plus indomethacin 5 mg kg^{-1} s.c. (■); indomethacin s.c. (□). The vertical bars indicate s.e. means (for each group $n = 5$).

Discussion

These experiments showed that captopril can potentiate inflammation in the rat caused by some, but not all, inflammatory stimuli. Oedema caused by intraplantar carrageenin, as assessed by either swelling of the hind foot or extravasation into the skin of Evans Blue, was increased after captopril. In addition the drug lowered the pain threshold of the oedematous site. These effects were probably due to increased bradykinin levels resulting from the drug's ability to block kininase II. Activity of the latter would be expected normally to reduce bradykinin levels during inflammation since kininase II is an ubiquitous en-

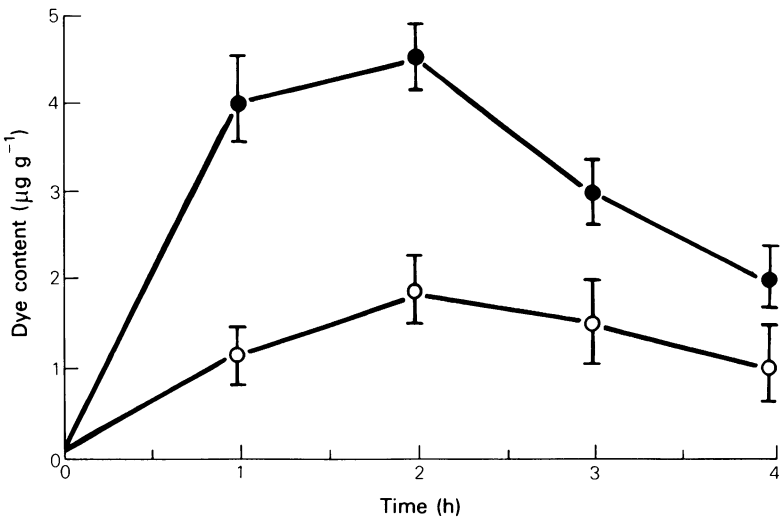

Figure 3 Mean Evans Blue dye content of the skin of the right hind feet at intervals after intraplantar injection of carrageenin. Controls (○); captopril 1 mg kg^{-1} s.c. (●). Each point represents the mean dye content of a group of 5. Vertical bars indicate s.e. means.

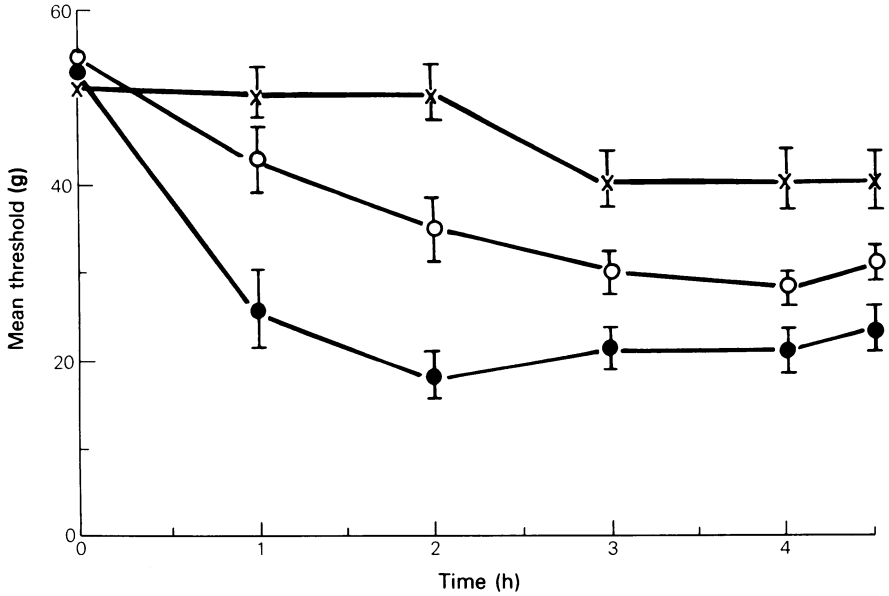

Figure 4 Mean pain thresholds of the right hind feet of groups of rats ($n = 8$). Saline s.c. and saline into the right foot (X); saline s.c. and carrageenin into the right foot (○); captopril 1 mg kg^{-1} s.c. and carrageenin into the right foot (●). Vertical bars indicate s.e. means.

zyme present in most tissues of the body including the plasma (Bakhle, 1980). Bradykinin has been shown to be a mediator of carrageenin inflammation in the rat (Di Rosa & Sorrentino, 1970). The subcutaneous dose of captopril used effectively blocked kininase II, being sufficient to potentiate 30 fold the vascular effects of bradykinin for the period of each experiment. Moreover, although other peptidases are known to inactivate bradykinin in the rat (Bakhle, 1980), the results indicated that kininase II may be most important in the hind paw in these circumstances. Oedema caused by bradykinin but not that caused by either histamine or prostaglandin E_2 was also potentiated by captopril which is a specific inhibitor of kininase II (Antonaccio, 1982).

Locally released prostanoids apparently contributed to the potentiation of inflammation by captopril. The increase in carrageenin-induced oedema after captopril was reduced after administration of the prostanoid synthesis inhibitor, indomethacin. Effects of increased bradykinin levels, resulting from inhibition of kininase II by captopril, would be expected to synergise with those of concomitantly released prostaglandins during carrageenin-induced oedema (Ferreira *et al.*, 1974). Indomethacin administration also reduced the potentiation of bradykinin-induced oedema by captopril. Bradykinin is known to act indirectly in some situations, by activating phospholipase A_2 causing synthesis and release of prostaglandins (Blackwell *et al.*, 1978). Nevertheless, indomethacin had no significant effect on oedema in response to bradykinin alone. The low concentration of bradykinin used in these studies was perhaps subliminal for production of effective local concentrations of prostaglandins. The higher concentrations of bradykinin achieved during inhibition of kininase II with captopril were evidently sufficient to cause stimulation of prostaglandin synthesis and release, since under these circumstances indomethacin had an inhibitory effect.

Wilkin *et al.*, (1980) have suggested that skin rash associated with captopril therapy in man is caused by inhibition of kininase II increasing previously subthreshold amounts of bradykinin to levels sufficient to cause an inflammatory effect. Our findings indicating that captopril can potentiate experimentally induced inflammation and that it probably does so through mechanisms involving increased tissue levels of bradykinin and prostaglandins, indirectly support the contention that pharmacological rather than immunological processes underlie this adverse effect.

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