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Effect of Converting Enzyme Inhibition with Captopril on Baroreflex Sensitivity

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Clinical and experimental data suggest that both Captopril and angiotensin II (AII) reduce baroreflex responsiveness, and the main action of this converting enzyme inhibitor (CEI) seems clear to suppress AII synthesis. The aim of this work is to investigate this striking similarity of effects. We have verified that CEI (4 mg/kg) originates tachycardia significantly lower ($P < 0.001$) than that produced in response to a similar hypotension elicited by an unspecific vasodilator: sodium nitroprusside (10-45 μg/kg min). CEI SQ 20881 has been reported to increase plasma vasopressin concentrations (AVP); this peptide is also known to modify baroreflex responses and has a small direct negative chronotropic effect. However, our determinations of AVP do not show any difference between the control group and the group treated with Captopril (4.78 ± 0.87 and 5.26 ± 0.19 pg/ml respectively). On the other hand, although CEI did not modify the rapid responses of heart rate (HR) to changes of mean arterial pressure (MAP), the decrease of MAP induced by nitroprusside was higher in the group treated with Captopril than in control group; it could mean a baroreflex ability decrease to buffer the hypotension. However, AII elicited a strong impairment of both rapid responses of HR and the buffering of hypotension produced by NP, these actions being suggested as centrally mediated. These results could indicate that the suppression of peripheral AII synthesis and therefore, the lack of pre- and postjunctional sympathetic potentiation owing to this hormone, is responsible for the absence of tachycardia under Captopril treatment.

Key words: Captopril, Converting enzyme inhibitors, Baroreflexes, Blood pressure.

Recent studies have shown that Captopril (SQ 14225), an orally active angiotensin I converting enzyme inhibitor, unlike many vasodilators, does not elicit reflex tachycardia when it lowers blood pressure. It appears that autonomic reflexes could be blunted by the drug (2, 11, 24). Several hypotheses have been used to explain this effect. Captopril abolishes the AII synthesis, and therefore, peripheral sympathetic responses could be blunted (3, 10, 23, 25). Moreover, AII inhibits the parasympathetic efferent tone,

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and thus CEI could increase the vagal chronotropic inhibition (2). On the other hand, the marked impairment of baroreflex sensitivity in hypertensive patients could mean that they have an extremely limited HR response to any decrement in blood pressure (24).

Surprisingly, baroreflex sensitivity is also impaired by AII, action that could be centrally mediated (9, 21). However, in these studies of baroreflexes, anesthetized animals have very often been used and it is well known that anesthetic agents elicit a strong impairment of cardiovascular reflexes both directly (12) and releasing important amounts of renin (14). It has been reported that converting enzyme inhibition with SQ 20881 increases AVP levels, action probably mediated by the increase of AI elicited by the drug (15). AVP is able to produce bradycardia, and may modify strongly baroreflex sensitivity (5, 20). These actions are mainly centrally mediated, but vasopressin also has a small chronotropic effect *in vitro* (13, 19). At the present, there are no data about the role of vasopressin on the hemodynamic actions of CEI.

The purpose of this work is to investigate, in normal conscious rats, the mechanism by which both AII and CEI seem to affect baroreflexes similarly, diminishing their responses to changes of blood pressure although their actions were expected to be antagonistic.

Materials and Methods

All experiments were performed on conscious male Wistar rats (280-330 g) in their home cage environment, 12 h after surgery. Rats were anesthetized with ether, and a catheter (1 mm Ø) was inserted into the femoral artery with the tip advanced into the abdominal aorta. A second double catheter (1 mm Ø) was inserted into the jugular vein, for drug administration. Heart rate (HR) and mean arterial pressure (MAP) were recorded with a 7754A Hewlett Packard, using two pressure amplifiers. The first was used to record MAP, and the second recorded the differential arterial pressure. HR was counted over 4 seconds for each determination. Continuous venous infusions were carried out using a peristaltic pump (Microperpex, LKB-Bromma, Sweden).

Blockade of the converting enzyme was performed with Captopril (Squibb), at a dose of 4 mg/kg, continuing with 1 mg/kg/h in infusion i.v. during 12 h.

Measurement of baroreflex function. — Baroreflex function was assessed in conscious rats by pharmacological increases of MAP using phenilephrine (PE) at doses of 1, 5, 12.5 and 25 μg/kg, and decreases of MAP using sodium nitroprusside (NP) at doses of 1, 5, 12.5 and 25 μg/kg. Graded doses of both were injected alternately in a volume of 50 μl each, and at least 10 min were allowed for stabilization between injections. Baroreflex line was calculated from peak responses of MAP and HR in each group using a least-squares linear regression model. The sensitivity of the reflex was determined by the slope of this line. A dose-response curve (doses of PE or NP-changes of MAP) was performed in every group. The increases or decreases of MAP produced by graded doses of PE and NP provide an index of the ability of baroreflex to buffer the vasodilation or vasoconstriction.

Statistics. — The slopes of regression lines HR- MAP were compared with Student's t test. Data of HR and MAP are expressed as means ± SE. Analysis of variance was used to evaluate measurements of HR and MAP. Statistical comparisons of doses of NP or PE- MAP curves were performed with the Student's t test.

Hemodynamic responses to Captopril, in comparison with sodium nitroprusside (N = 6 and n = 6). — The hypotension and tachycardia elicited by Captopril (4 mg/kg, i.v.) were compared to hemodynamic responses produced by NP (10-45 μg/kg/min). NP infusion rate was regulated to obtain a decrease of MAP similar to the hypotension elicited by the former drug. Both experiments were performed with two different groups of rats.

Baroreflex responses in the presence of intravenous infusion of Captopril (n = 7), compared to a control, untreated group (n = 12). — Baroreflex function was determined after a 12-hour period of CEI infusion at a rate of 1 mg/kg/h (0.1 ml/h), and then compared with an untreated group.

Baroreflex responses in presence of intravenous infusion of Angiotensin II (n = 7). — Baroreflex sensitivity was tested after a 30 min period of intravenous infusion of AII at a rate of 10 ng/kg/min, as previously described.

Plasma AVP levels under Captopril infusion compared to a control group (n = 5 and n = 6). — Treatment with Captopril (4 mg/kg I.V., continued by infusion at a rate of 1 mg/kg/h I.V. during 30 min) was compared to a group infused with NaCl 0.9 %, at the same rate. Vasopressin was determined by radioimmunoassay after an extraction procedure from plasma using ethanol 100 % (−20° C); after centrifugation, the supernatant was air-dried and reconstituted with phosphate buffer pH 7. The recovery was 81 ± 2.08 %. RIA determination of AVP was in principle performed according to the method described previously (17). The rabbit antiserum was kindly provided by Ciba, proceeding from Dr. F. Lishajko (Karolinska Institute). As regard cross reactions between AVP and its analogues 8-arginine vasotocin, lysine vasopressin and oxytocin, the binding affinity of these analogues to antiserum were 22, 3.1 and 0.004 % respectively in comparison to that of AVP (17). The detection limit of RIA was 0.9 pg/ml, the coefficient of variation (CV) intraassay was 8.29 %, and the CV interassay was 11.6 %.

Results

Comparison between the tachycardia elicited by Captopril and nitroprusside administration (fig. 1). — Non significant differences between the hypotension produced by both drugs were observed, but CEI originated tachycardia significantly lower than NP ($P < 0.001$).

Differences of baroreflex sensitivity between the control group and the group treated with Captopril. — Intravenous administration of CEI during 12 h did not significantly change the slope of the

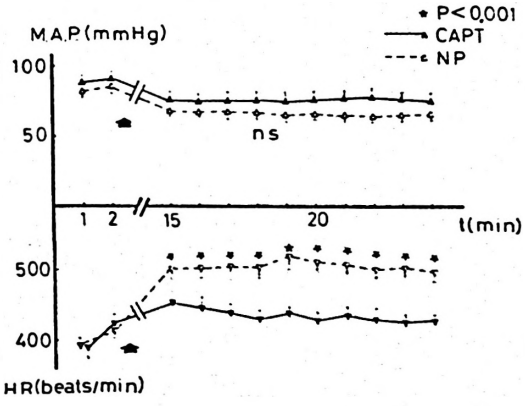

Fig. 1. *Comparison between the tachycardia elicited by Captopril (CAPT) and sodium nitroprusside (NP), in response to similar decreases of mean arterial pressure (MAP).*
n.s.: not statistically significant. The arrows show the beginning of Captopril (4 mg/kg, in bolus i.v., continued by an infusion at a rate of 1 mg/kg/h) as well as nitroprusside administration (10-45 μg/kg/min).

regression line MAP- HR compared to control group (fig. 2).

The hypotension elicited by the administration of NP (bolus i.v.) was significantly higher in the group treated with Captopril compared to control group, at the doses of 5, 12.5 and 25 μg/kg of NP.

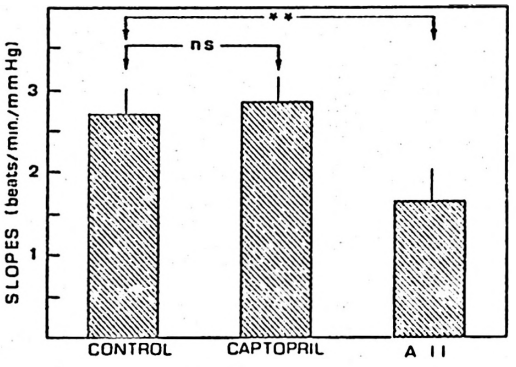

Fig. 2. *Comparison between baroreflex sensitivity (indicated by the slope of regression line) in the three experimental groups.*
Untreated group (control): (y = 20.69-2.69x, r = −0.92): Group infused during 12 h with Captopril (1 mg/kg/h, [y = −4.14-2.84x, r = −0.94]), and group infused with angiotensin II (AII) 10 ng/kg-/min during 30 min (y = −4.04-1.64x, r = −0.86) n.s.: not statistically significant. **: P < 0.001.

There were no differences between these two groups in respect of the pressor responses to graded doses of PE (table I).

Difference of baroreflex responses among the group treated with AII infusion, the control group and the group treated with Captopril. — Administration of AII (10 ng/kg/min i.v.) elicited a fall in the slope of the regression line MAP- HR compared to control group (P < 0.001, fig. 2).

The hypotension produced by the injection of graded doses of NP was significantly higher in the group infused with AII compared to control group at the doses of 25 (P < 0.001), 12.5 (P < 0.001) and 5 μg/kg of NP (P < 0.05). There were no differences between these two groups in respect to the pressor responses to graded doses of PE. There were no statistical differences between the groups treated with Captopril and AII either in blood pressure falls elicited by injection of NP or in respect to the pressor responses to PE (table I).

Plasma AVP levels under Captopril treatment. — Plasma AVP was not significantly different in the group treated

Table I. *Effect of different doses of phenilephrine and nitroprusside on arterial pressure in the three experimental groups.*
Control: untreated group, group infused with Captopril (1 mg/kg/h) during 12 h, and group infused whith angiotensin II (10 ng/kg/min) during 30 min. *: P < 0.05; **: P < 0.001. Values are expressed as means ± SD.

Doses (μg/kg)	Changes of mean arterial pressure Control	Captopril	Angiotensin II
PHENILEPHRINE			
1	14.1 ± 4.98	9.71 ± 7.78	12.28 ± 4.4
5	29.3 ± 4.82	23.28 ± 6.92	27.28 ± 5.7
12.5	33.3 ± 4.94	37.14 ± 8.25	36.14 ± 6.5
25	38 ± 6.26	44 ± 11.4	43.14 ± 6.7
NITROPRUSSIDE			
1	−6.2 ± 3	−5.8 ± 4.3	−9 ± 2.9
5	−12.8 ± 3.7	−21.28 ± 4.9(*)	−20.85 ± 6.9 (*)
12.5	−22.8 ± 7.5	−30.7 ± 6(*)	−35.42 ± 7.3(**)
25	−31.6 ± 5	−39.57 ± 13(*)	−42.42 ± 8.8(**)

with Captopril, compared to the control group (5.26 ± 0.19 versus 4.78 ± 0.87 pg/ml, respectively).

Discussion

The clinical use of CEI has shown a blood pressure fall without reflex tachycardia (2, 11, 24). Cardiac output increased but contrary to the effects of other vasodilators, it was due to a higher stroke volume with an unchanged heart rate (11). This absence of tachycardia has been attributed to a blunting of baroreflex sensitivity present in hypertensive patients before they are treated with CEI (24). However, in normal conscious rats a significantly lower tachycardia with Captopril in comparison to NP (fig. 1) it is observed, and similar results having been obtained in studies carried out in normotensive humans (11). Thus, the drug seems to blunt by itself those baroreflex responses of HR to changes of MAP. Paradoxically, it is well known that AII, the synthesis of which is suppressed by CEI, elicits an impairment of baroreflex sensitivy (9, 10) diminishing both sympathetic and parasympathetic responsiveness of baroreflexes, an action that maybe is centrally mediated (9, 10, 18).

SQ 20881 has been reported to increase AVP levels in normal rats, but this action has not been demonstrated in nephrectomized rats (15). Thus, the AI raised by CEI in plasma appears to be able to stimulate the AVP release, which could be important as AVP has been shown to have a direct negative chronotropic effect *in vitro* (13, 19), and it could explain the absence of tachycardia observed under Captopril treatment (2, 11, 24). However, CEI has been found not to change baroreflex sensitivity, and vasopressin is known to increase it strongly (5, 20). In addition, our determinations of plasma AVP values do not show an increase in the group treated with CEI in respect to controls. Therefore, Captopril, at the dose used in this study, is not able to release vasopressin, and this «releasing action» of CEI cannot explain the absence of tachycardia.

AII may increase sympathetic outflow acting on the circumventricular organs of the central nervous system (21, 8, 16), and the hormone has been suggested to also inhibit the parasympathetic activity. The main action of Captopril is to suppress AII synthesis and, thereby increase the parasympathetic efferent tone (2). Our results show that the treatment with CEI does not significantly change the rapid, mainly vagal (4) responses of HR to changes of MAP (fig. 2), thus rendering impossible for us to support this hypothesis. Similar results were found by BERECEK *et al.* The only remaining explanation to the absence of tachycardia could be therefore, a decrease in the sympathetic response of baroreflexes.

As previously described (9, 10), AII has been found to significantly impair baroreflex sensitivity, and this action has also been suggested to be centrally mediated (21, 9). However, Captopril only elicits a decrease on the buffering of the hypotension (table I), which could be originated by the low tachycardic response induced by CEI. It is known that converting enzyme inhibition has some effects upon both pre- and postjunctional peripheral sympathetic responses in rats. We agree with other investigators who have shown that Captopril-induced attenuation of pressor responses to norepinephrine as well as sympathetic stimulation were prevented by bilateral nephrectomy, and restored by AII infusion (3, 10, 23). Furthermore, others have found that the blood pressure decrement in response to alpha-blockade was reduced by CEI, indicating a decrease of sympathetic tone elicited by the drug (24).

Therefore, AII suppression elicited by Captopril (and not an increase of AVP

levels) is suggested as the main cause of lowered tachycardia in response to hypotension. These results also indicate that, in conscious unrestrained rats, the renin-angiotensin system does not take any part in baroreflex function, since the converting enzyme blockade does not affect baroreflex sensitivity as AII does. Moreover, the effect of Captopril on sympathetic activity may indicate that in normal conditions the renin-angiotensin system regulates the sympathetic control of vascular walls. This action could be mediated by the generation of AII in plasma or more probably by its generation in the vascular wall (6, 7, 22).

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Resumen

Datos clínicos y experimentales sugieren que tanto el Captopril como la Angiotensina II (AII) reducen la respuesta barorrefleja, a pesar de que ambos tienen acciones antagónicas. Se verifica experimentalmente que el Captopril produce una taquicardia significativamente menor que otro vasodilatador, el nitroprusiato, ante hipotensiones similares. Esta acción no es producida por un incremento de los niveles plasmáticos de vasopresina, que posee ligera acción cronotrópica negativa. Los resultados indican que el tratamiento con Captopril disminuye la capacidad del barorreflejo para tamponar la hipotensión. Ello podría indicar que la supresión de la síntesis de AII disminuye la potenciación que causa a nivel pre y postsináptico en la actividad simpática, originando así una menor respuesta taquicárdica ante la hipotensión.

Palabras clave: Captopril, Inhibidores de enzimas convertidoras, Barorreflejos, Presión sanguínea.

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