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Difference Between Intracarotid and Intravenous Infusions of Angiotensin II on Baroreflex Sensitivity and Vasopressin Release in Conscious Rats

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F. J. FENOY, M. UBEDA, J. GARCIA-ESTAÑ and T. QUESADA. *Difference between Intracarotid and Intravenous Infusions of Angiotensin II on Baroreflex Sensitivity and Vasopressin Release in Conscious Rats.* Rev. esp. Fisiol., 43 (3), 355-360, 1987.

A carotid infusion of angiotensin (AII) (10 ng/kg/min) has been found to increase significantly higher mean arterial pressure (MAP) and produces significantly lower bradycardia than AII intravenous infusions at the same dose and rate. Besides, i.v. administration of AII elicits greater impairment on baroreflex sensitivity than carotid infusion of AII does. On the other hand, vasopressin vascular receptor blockade did not modify the baroreflex sensitivity either in the carotid or in the i.v. infusions of AII, and plasma AVP measurements did not change significantly in any group. It clearly indicates that neither AVP nor baroreflex impairment plays any role on the pressor action of AII intracarotid infusions at a low dose. The present results further suggest that baroreflex impairment in rats may unlikely be located in the region irrigated by the carotid artery.

Key words: AVP, Baroreflex sensitivity, Carotid AII.

Baroreflex responsiveness is well known to be reduced in renovascular hypertension (5, 17) while i.v. angiotensin II (AII) infusions have been found to elicit the same actions (5, 6, 12). AII also produces an increase in arterial pressure which may partially be mediated by the central nervous system (3). FERRARIO *et al.* (3) have shown that intravertebral infusions of AII in dogs produced rises in blood pressure considerably higher than those by intravenous route, their results indicating that intravertebral AII increases sympathetic nerve activity. In rats, the pressor response produced by intracarotid administration of AII is significantly higher than that elicited intravenously (3, 10). This action has been located in/near the circumventricular organs of the anteroventral third ventricle region (3), and is blocked by central saralasin (10). Moreover, the reflex sympatho-inhibition that usually accompanies an increase in blood pressure was not observed after AII intracarotid administration (16).

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However, it remains unclear whether these central pressor responses to AII in rats originate from stimulation of central sympathetic neurons or from central impairment of baroreflex responses (16).

Since AII can release vasopressin (AVP), such a AVP-release could be higher from intracarotid than from i.v. infusions of AII (13). The AVP pressor contribution to carotid injections of AII has not been examined previously (10). This may be important because of the great vasoconstrictor ability of AVP acting on a cardiovascular system with its baroreflexes blunted by AII.

The purpose of this work is to investigate whether the enhanced pressor action of AII, when infused into the carotid artery, is partly due to a higher blunting of baroreflex responsiveness and/or to a possible increase of vasopressin release.

Materials and Methods

Male Wistar rats, weighing 280-330 g, were used in this study. All experiments were performed on conscious rats in their home cage environment, 12 h after surgery. Rats were anethestized with ether, and a catheter was inserted into the femoral artery with the tip advanced into te abdominal aorta. A second double catheter (1 mm ∅) was inserted into the femoral vein, for AII infusion and drug administration.

In the carotid infusion group, the femoral artery and vein were also catheterized, using poliethylene tubing (1 mm ∅), and a third catheter (0.7 mm ∅) was placed into the right brachial artery, with the tip advanced to the bifurcation of the subclavia and the right common carotid artery. As it has been previously described (4), this technique facilitated the central infusion of AII without interfering with the blood supply to the brain.

Mean arterial pressure (MAP) and heart rate (HR) were measured with a Hewlett-Packard system (Transducer 1290A, Amplifier 8805C and Recorder 7754A). Mean and differential arterial pressure were recorded, and HR was counted over at least 4 s for each determination. Continuous venous and arterial infusions were carried out using a peristaltic pump (Microperpex, LKB-Bromma, Sweden).

AII (Hypertensina, Ciba-Geigy) and specific vasopressin vascular receptor antagonist (AVP-a, 1-β-Mercapto-β, β-Cyclopentamethylene propionic acid), 2-(*o*-Methyl tyrosine) arginine-vasopressin (BACHEM), were also used.

Measurement of baroreflex function. — Baroreflex function was assessed in conscious rats by pharmacological increases of MAP with phenylephrine (PE, Cusi) at doses of 1, 5, 12.5 and 25 μg/kg, and by decreases sodium nitropruside (NP, Fides) at the same doses. Graded doses of both were injected i.v. alternately. At least 10 min were allowed between increasing doses for stabilization. Peak responses in MAP and HR for each injection were tabulated. Baroreflex slope 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.

Statistics. — The slopes of regression lines HR — MAP were compared with the Student-t test. Data of HR and MAP are expresed ± SE. Analysis of variance was used to evaluate HR and MAP measurements.

Baroreflex responses to PE and NP of control untreated group (n = 7). — The MAP was modified with graded injections of PE and NP. The volume of each dose was 50 μl. The experiment was started 12 h after surgery, followed by a period of 45 min for hemodynamic stabilization.

Baroreflex responses in presence of i.v. infusion of AII (10 ng/kg/min). Effect of AVP-a (n = 7). — The i.v. infusion of AII was started 30 min before the administration of PE and NP, and pre- and postinfusional values of HR and MAP were measured as previously described. The infusion of AII was not interrupted during injections using a venous double catheter. Four hours later, the baroreflex function was checked in presence of combined treatment of AII (10 ng/kg/min) and AVP-a (10 μg/kg, i.v.), in the same animal.

Baroreflex responses in presence of i.v. PE infusion (n = 7). — Baroreflex sensitivity was tested in presence of a PE venous infusion (200-350 ng/kg/min), at a dose adjusted to increase MAP 5-10 mmHg (similar to the increase obtained with AII). This experiment was used as a control of i.v. AII infusion.

Baroreflex responses in presence of intracarotid AII infusion. Effect of AVP-a (n = 7). — Baroreflex responsiveness was studied in presence of an intracarotid AII infusion (10 ng/kg/min) at the same rate and dose used in the i.v. infusion group. Four hours later, baroreflex sensitivity was examined in presence of combined treatment of intracarotid AII (10 ng/kg/min) and AVP-a (10 μg/kg, i.v.).

Measurement of Arginine-Vasopressin plasma levels (AVP). — Three groups of rats were infused with NaCl 0.9% (1 ml/h i.v., n = 6), AII i.v. (10 ng/kg/min, 1 ml/h, n = 5) and intracarotid AII (10 ng/kg/min, 1 ml/h, n = 5) respectively, during a 30 min period; then, a blood sample (1 ml) was obtained through the arterial catheter. Plasma AVP was determined by RIA 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 (11). The rabbit antiserum was kindly provided by CIBA (Dr. F. Lishajko, Karolinska Institut). As regard cross reactions between AVP and its analogues 8-arginine vasotocin, lysine-vasopressin and oxytocin (Sigma) the binding affinity of these analogues to antiserum were 22, 3.1 and 0.004% respectively in comparison to that of AVP (11). The detection limit of RIA was 0.9 pg/ml, and the coefficient of variation (CV) intraassay was 8.29%, and the CV interessay was 11.6%.

Results

Comparison between the hemodynamic alterations elicited by intracarotid and intravenous AII infusions (fig. 1). — Intravenous infusion of AII produced a significantly lower increase of MAP, and a significantly higher bradycardia ($p < 0.001$) than intracarotid infusion of AII.

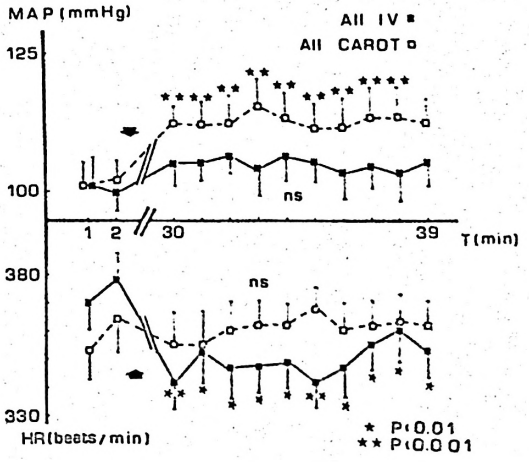

Fig. 1. *Hemodynamic responses elicited by intravenous (AII i.v.) and intracarotid (AII CAROT) AII infusions, both at a dose of 10 ng/kg/min.* The arrows show the beginning of the infusion. Statistical comparisons were performed between pre-and postinfusion values in each group.

Action of AII i.v. on baroreflex sensitivity. Effect of AVP-a. Comparison with PE i.v. infusion. — Baroreflex sensitivity was significantly lower ($p < 0.001$) in the group treated with AII i.v. than that in the control group (1.64 versus 2.69 beats/min/mmHg, fig. 2). The regression lines were $y = 20.69-2.69x$ ($r = -0.92$) in the control untreated group, $y = -4.04-1.64x$ ($r = -0.86$) in the group infused with AII i.v., and $y = 32.21-2.91x$ ($r = -0.94$) in the group treated with PE i.v. The group infused with PE i.v. (200-350 ng/kg/min) was no statistically different from the control group (fig. 2).

AVP-a did not change significantly the slope of the regression line in the group treated with AII i.v. The regression line of this group infused with AII i.v. and AVP-a was $y = 19.66-1.68x$ ($r = -0.9$).

Action of intracarotid AII on baroreflex sensitivity. Effect of AVP-a. — Baroreflex sensitivity was significantly higher in the intracarotid infusion group compared to the i.v. AII infusion group (1.64 versus 1.97 beats/min/mmHg, fig. 2). The regression line of the group infused with intracarotid AII was $y = 14.85-1.97x$ ($r = -0.91$).

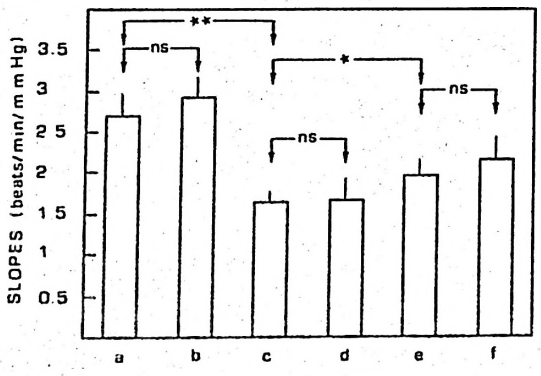

Fig. 2. *Slopes of regression lines of the groups control untreated (a), and the groups infused with PE i.v. (b), AII i.v. (c), AII i.v. +AVP-a(d), AII intracarotid (e), and AII intracarotid×AVP-a (f).* ns: not statistically significant; *: $p < 0.05$; **: $p < 0.001$.

AVP-a did not significantly change the baroreflex sensitivity in the intracarotid AII infusion group. The regression line of the group treated with intracarotid AII and AVP-a Was $y = 15.78-2.15x$ ($r = -0.93$).

Determinations of AVP. — AVP was not statistically different in the groups infused with NaCl 0.9% (4.782 ± 0.879 pg/ml), AII i.v. (7.147 ± 1.337 pg/ml) and intracarotid AII (7.09 ± 0.834 pg/ml).

Discussion

Intracarotid infusion of AII has been found to elicit a higher rise in blood pressure than the same dose of AII infused i.v., confirming results previously described (3, 10, 16). Furthermore carotid infusions elicited decreases of HR significantly lower than i.v. infusions, despite its higher increases in blood pressure. There are two possible explanations: either the impairment of baroreflex control is higher in the carotid infusion group, and the blood pressure rises without any change in HR; or the increase in the sympathetic outflow elicited by the intracarotid administration raise the blood pressure and increase heart rate. The former possibility is unlikely, since the present results show that baroreflex sensitivity is higher in the AII carotid infusion as compared to the i.v. AII infusion group (fig. 2), and its hemodynamic effects are probably due to increased sympathetic efferent tone. Baroreceptor impairment by AII has been shown mainly in dogs (2, 12, 17), rabbits (5, 15) and cats (6, 14). Since the central effects of AII appear so different in the rat, on one hand, and in the cat, dog an rabbit, on the other (3), it seems unlikely that results obtained in the latter species bear any relationship on rat mechanisms. At present, carotid AII infusions are known

to increase blood pressure in excess of systemic administration in rats, by acting on the AV3V region. However, in the pressor action of AII carotid infusions in rats and cats), it has been impossible to distinguish between stimulation of central sympathetic neurons and central attenuation of baroreflexes (16). Our data strongly indicate that baroreflexes are not involved and that an increased sympathetic output could be the main cause of this AII central action in rats, as it happens in dogs. Nevertheless, a third possibility exists: the AII induced release of AVP could be higher in the carotid than in the i.v. AII infusion group (3, 13). It could explain the enhanced pressor response and the higher baroreflex sensitivity in the carotid infusion group, since AVP is a potent vasoconstrictor agent and sensitizes baroreflexes (2, 15). At present, the role of vasopressin on the pressor response of AII carotid infusions remains unclear (10). The present results show that AVP-antagonist did not modify significantly the baroreflex responsiveness either under carotid or i.v. AII infusions (fig. 2). However, it has been suggested that the effect of AVP on baroreflexes could be mediated through a vasopressin receptor similar to the vasopressin renal receptor, this AVP-antagonist being specific of AVP vascular receptor (7). To resolve this problem, AVP was determined by radioimmunoassay and was not found to increase either in the carotid or in the i.v. AII infusion group, confirming the results obtained by pharmacological blockade and coinciding with results from other authors who found that only high doses of AII (about 250 ng/kg/min) are able to release vasopressin in rats (13). AVP has, therefore no importance on either pressor actions of AII carotid infusions, or its actions on baroreflexes.

In rats, the enhanced pressor response to intracarotid infusions of AII is abolished by electrolytic lesion in the anteroventral third ventricle tissue (AV3V), the circumventricular nuclei in this area probably being the action site of AII (3). However, the location of the AII action site on baroreflexes is unknown (5, 16). The present results strongly suggest that it is not probably located in the region irrigated by the carotid artery; this action site, if central, should be different from the site responsible for the increase in sympathetic outflow, as previously pointed out in cats (14).

Summing up the AII induced release of vasopressin has been shown to have little bearing on either the hemodynamic effects or the baroreflex impairment elicited by an intravenous or intracarotid infusion of AII at a low dose. Furthermore both actions of AII, increasing sympathetic outflow and blunting baroreflex sensitivity, could be two different effects located in separate sites, probably within the Central Nervous System.

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Resumen

La infusión de Angiotensina II (AII) intracarotídea (10 ng/kg/min) en rata aumenta significativamente la presión arterial media (PAM) y produce una bradicardia significativamente menor que la infusión venosa. Además, la infusión i.v. de AII reduce la sensibilidad barorrefleja significativamente más ($p < 0,001$) que la infusión intracarotídea. Por otra parte, el bloqueo farmacológico de los receptores vasculares de la vasopresina no produce cambios ni en la respuesta del reflejo barorreceptor en ninguno de los dos grupos, ni en la medición de la vasopresina por radioinmunoanálisis, lo que indica que ni la vasopresina ni la disminución de la sensibilidad del barorreflejo tienen importancia en la acción presora de la AII intracarotídea, en ratas. Estos resultados apoyan la idea de que, en la rata, la acción de la AII sobre el barorreflejo, probable-

mente, no está localizada en la zona encefálica irrigada por la arteria carótica.

Palabras clave: Angiotensina II, Vasopresina, Sensibilidad barorrefleja.

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