Effects of clonidine on aortic elastic modulus and aortic stress in anesthetized rabbits[1]

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Summary. Results from experiments performed in anesthetized rabbits suggest that, after clonidine injection, the aortic elastic modulus tends to remain constant by adaptative changes in the aortic wall which override the concurrent geometrical changes to maintain a level of elasticity consistent with the value of aortic stress.

The antihypertensive effect of clonidine has been attributed to a centrally-mediated reduction of sympathetic outflow[2-4] via a possible activation of the central pathway of the baroreceptor reflex[5]. For Aars[6], the increase in baroreceptor discharge observed after clonidine results from a dilatation of the aortic wall probably due to an adrenolytic effect exerted directly upon muscle fibers. More recently, Lombardi et al.[7] have suggested that clonidine can affect the circulation by an action independant of any depressant effect on sympathetic discharge. Their results lend further support to clonidine having a direct action on vascular smooth muscle.

The present study was undertaken to check whether a direct effect of clonidine on aortic blood pressure-diameter and elastic modulus-aortic stress relations could contribute to the hypotensive action of the drug.

Methods. Experiments were performed in 8 New Zealand white rabbits anesthetized with i.v. pentobarbital sodium. Aortic blood pressure was monitored in the proximal part of the abdominal aorta by means of a saline-filled catheter introduced through the right carotid artery and connected to a pressure transducer. A catheter was inserted into the right jugular vein for drug injections or blood removal. External aortic diameter (EAD) of the proximal part of the abdominal aorta was measured by means of an ultrasonic dimension technique similar to that used in sheep[8] and in cats and dogs[9]. Calculation of aortic elastic modulus (AEM) and midwall aortic stress (MAS) was performed according the method previously described in sheep[8]. For each animal, normal relationships between the different variables were established during control runs by altering blood pressure by either moderate stepwise removal and reinfusion of blood or by abrupt mechanical obstruction of the distal part of the abdominal aorta by means of an implanted occluder. An identical procedure was used after clonidine injection, each animal being its own control. Mean aortic blood pressure (MBP) being reduced by clonidine, the direct comparison between the values of mechanical vascular parameters measured before and after drug was inadequate. Thus, in order to evaluate the true effects of the drug on these parameters, we have selected the values obtained at 'normalized pressures' (MBP range: 85-105 mm Hg). This was achieved by selecting the values measured before drug during moderate stepwise bleeding (n = 36 measures in the 8 animals), and after drug, during mechanical obstruction of the distal part of the aorta (n = 35 measurements in the 8 animals). Clonidine chloride was dissolved in saline and given as a bolus at a dose of 25 μg kg^{-1} in 30 sec. Measurements of diameter and mechanical parameters were done at the peak of the hypotensive phase induced by clonidine. Mean values ± SEM were given. Differences between values measured before and after drug were calculated (Student's t-test) only for experiments in animals with resting control MBP above 80 mm Hg. Significance was taken to be a p-value of 0.05 or less.

Results. As expected from previous observations, clonidine injection produced a primary brief pressor effect (MBP = 116.5 ± 2.0%, $p < 0.001$) followed by a fall lasting up to 15-20 min. Peak effect (MBP = 79.7 ± 2.1%, $p < 0.001$) was observed between 5 and 15 min after drug injection. The brief primary hypertensive phase was not observed in 2 experiments but the hypotensive I was still present. In these 2 cases, the effects of clonidine upon vascular mechanical parameters were less important. Nevertheless, for the 8 rabbits, when compared at normalized pressure, the changes in diameter, aortic stress and elastic modulus were significant (table).

Individual relationships between hemodynamic and mechanical parameters calculated for the 8 animals show that, after clonidine injection, the instantaneous relationships between blood pressure and diameter, aortic stress and elastic modulus, respectively, were shifted towards lower pressure at any given diameter, stress and elastic modulus. However, by contrast with the above mentioned relationships, values of elastic modulus, calculated as a linear function of the stress, were slightly but not significantly higher after clonidine than before drug when compared over a common stress range.

It is important to mention that all the individual relationships between the different variables were well described ($r > 0.95$) by exponential or linear curves.

Conclusions. The present experiments show that acute i.v. administration of clonidine in anesthetized rabbits induced an increase in the abdominal aortic diameter and elastic modulus when compared at common blood pressure level. As supposed by Aars[6], clonidine seems to induce a relaxation of aortic smooth muscle. Our data indicate that aortic diameter, aortic stress and aortic elastic modulus were increased when blood pressure level was normalized by aortic constriction under drug. It is likely that pressure-diameter relationship changes observed in the present study were primarily reflecting the intrinsic passive elastic properties of the vessel. It might be supposed that the increase in diameter caused by relaxation of aortic smooth muscle by increasing aortic wall stress allows for compensation and even inversion of the decrease in wall stress resulting from the decrease in blood pressure. When compared over a

Effects of clonidine (25 μg kg^{-1}) on vascular parameters observed at normalized pressures (range: 85-105 mm Hg)

	Mean blood pressure (mmHg)	Mean external aortic diameter (mm)	Midwall aortic stress (10^5 dynes/cm^2)	Aortic elastic modulus (10^6 dynes/cm^2)
Control (n = 36)	97.6 ± 0.9	3.33 ± 0.06	8.93 ± 0.41	2.43 ± 0.21
Clonidine (n = 35)	94.9 ± 1.3	3.77 ± 0.04	12.51 ± 0.83	3.66 ± 0.21
p	NS	< 0.001	< 0.001	< 0.001

p-values refer to differences from control; NS, not significant; n, is the number of measurements in the 8 animals.

common stress range, the aortic elastic modulus was not significantly different before and after drug. One possible interpretation is that the 'operating level' of the aortic elastic modulus tends to be maintained constant by adaptative changes in the aorta which override the concurrent geometrical changes to maintain a level of elasticity consistent with the value of aortic stress, probably associated with the development of an optimal load for the left ventricle.

On the other hand, cardiac receptors signalling in unmyelinated vagal afferents are of interest. Their activation can cause an increase of vagal tone on the heart and an inhibition of sympathetic activity[10]. The possibility of an action of clonidine upon cardiac receptors was pointed out in a recent work of Lisander and Wennergren[11]. The authors concluded that clonidine could activate vago-vagal reflexes, probably emanating from the heart and secondary to peripheral hemodynamic changes.

In rabbits with cervical spinal cord transection, Petty et al.[12] observed that clonidine caused a significantly greater pressor effect but the hypotensive phase was completely abolished. The authors suggested that the potentiation and prolongation of the initial pressor effect may represent the direct effect of the drug on peripheral α-adrenoceptors unopposed by a central hypotensive action. The present data lend further support to this hypothesis since we observed that the increases in aortic diameter, aortic stress and elastic modulus were less important when the hypertensive phase was absent.

In conclusion, the principal site of the hypotensive action of clonidine seems to be in the CNS but an additional direct effect of the drug upon vascular smooth muscle cannot be discarded. Our results show that aortic diameter, aortic stress and elastic modulus were increased after clonidine. This direct peripheral action of the drug could play, in addition to a central effect, an important role in modifying the activity of vascular and/or cardiac receptors and thus influencing a) the central components of the baroreceptor reflex arc, b) the preganglionic and postganglionic sympathetic nerve activities, c) the parallel changes in stiffness and elastic modulus of the aortic wall.

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Monoamine oxidase activities in human brain microvessels[1]

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Summary. Microvessels can be easily isolated from human brain samples obtained at autopsy. Human frontal cortex MAO type A and B activities are similar in microvessel and microvessel-free preparations. In microvessels, enzyme activities and the ratio of MAO type A to type B vary among the areas studied and could selectively regulate the passage of certain amines through the blood vessel wall.

Recently, methods have been developed allowing for the isolation of microvessels from various animal brains[3-5]. These preparations have been used to study a variety of metabolic processes and enzymes[6-8], including monoamine oxidase (MAO)[9-11]. It was observed that rat brain microvessels exhibit a 3 times greater activity of MAO than microvessel-free brain homogenates[11] and that they contain type A and B activities although the final ratio of A:B is still uncertain[10].

The purpose of this study was to determine if microvessels can be prepared from human brain samples obtained at autopsy, if human microvessels show MAO activities similar to those found in rat brain microvessels and if differences in MAO activities can be detected among brain regions. It was decided that human autopsy material could be used for the study since MAO in rat brain was shown to be rather stable post mortem[12,13]. The substrates used to obtain preliminary information on MAO activities were phenylethylamine (PEA), a general type B substrate in rat brain[14], and serotonin (5-HT), a general type A substrate[15].

Male Wistar rats (250–300 g) were obtained from Perfection Breeders, Douglasville, PA. Human brain samples were obtained between 10–15 h post mortem from individuals free of brain or major vascular disease. ^{14}C-PEA and ^{14}C-5-HT were purchased from the New England Nuclear Corporation. Microvessels were prepared according to the method of Mrsulja et al.[5]. Microvessel-free homogenates were obtained by using the method of Lai et al.[11]. MAO activity was measured basically as described by Wurtman and Axelrod[16].

V_{max} was calculated by linear regression analysis using Eadie-Hofstee parameters[17].

Phase contrast photomicrographs showed that human microvessels can be isolated relatively pure and seem to be similar in appearance to microvessels obtained from whole rat brain[3,11].

MAO type A (5-HT) and type B (PEA) activities of rat and human brain homogenates and microvessels are shown in the table. In contrast to Lai et al.[9,10], who found most of the MAO activity in microvessels, we find similar MAO activities in whole brain homogenate, microvessel-free homogenate and microvessels. This indicates that MAO activities might be more equally distributed between microvessels and microvessel-free fractions. The difference between our data and those of Lai et al.[9,10] could be due to the procedure used to isolate microvessels; we use the 'fractionation'