Adrenergic Receptor Blockers on Pressor Activity of Incubated Human Plasma (33581)

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An albumin or a protein bound molecule accounts for the pressor activity of the plasma incubated at normal pH and at a temperature of 38° for several hours (1–4). The mechanism of its pressor action is still unknown, the question arises whether the pressor effect produced by this substance is due to the release of plasmakinins or other unknown polypeptide. Bradykinin (4) and kallidin (5), normally having vasodilator properties, produce in the ganglion-blocked rat a rise in blood pressure. This pressor effect is due to the release of catecholamines (6–9). Other experiments have demonstrated also that the vasoconstrictor effect of angiotensin is in part mediated through the sympathetic nervous system (10–12).

It was considered of interest to investigate whether the hypertensive effect obtained with incubated plasma or its active fraction was the result of the liberation of catecholamines from the adrenal medulla and/or from adrenergic nerve endings. In order to prevent catecholamines liberation, adrenalectomy and α and β adrenergic receptor blocking substances were used.

Methods. The experiments were conducted in white rats of both sexes, ranging in weight from 200 to 320 g. Most of the rats which were under ether anesthesia were nephrectomized 16 hr before testing the drugs. For this purpose the animals were anesthetized with intraperitoneal injection of dialylbarbituric acid–urethane solution (Dial, Ciba, Basel) in a dose of 0.1 ml/100 g of body weight. This preparation in this dosage produces in the nephrectomized rat a lowering of the blood pressure which remains stable for several hours. Bilateral adrenalectomy, when necessary, was performed immediately before the experiments by a dorsolumbar approach.

The blood pressure was measured by introducing a small cannula in the carotid artery and the cannula was connected with a manometer (Hürtle type) calibrated with a mercury manometer. Heparin was given immediately before cannulation. A polyethylene tubing introduced in the femoral vein permitted the administration of the different substances, either by single injections or by slow infusion.

Valyl 5 angiotensin amide II (Ciba), synthetic brandykinin (Sandoz), adrenaline HCl, diluted in 0.9% saline solution were used as standards, and injected in a volume of 0.1 or 0.2 ml followed by 0.05 ml of saline solution.

The other drugs employed were: phentolamine HCl (Regitine, Ciba), azamethonium dichloride (Pendiomid, Ciba) and propranolol (Inderal I.C.I.). All these drugs were administered intravenously in a volume of 0.1 ml 0.9% saline solution at the doses specified below.

Incubation of plasma. Blood was obtained by venous puncture from healthy adult persons. It was collected directly through a short polyethylene tubing into siliconized, sterilized, and graduated glass bottles fitted to a refrigerated centrifuge. Although no significant differences were found using plasma or serum, it was felt that for this study it was essential to use silicone-treated glassware to minimize the risk of producing artifacts by surface activated plasma enzymes.

Enough heparin to get a final concentration of 0.5 or 2 IU/ml, when mixed with the blood, was introduced in the bottle before bleeding. A rapid mixture of the blood with the anticoagulant was facilitated with gentle agitation. Immediately after centrifuging for 30 min in the cold, the plasma was transferred to another sterilized, siliconized bottle by aspiration. Only the upper layer of plasma was used to prevent the presence of blood

cells. No toluene or any other bacteriostatic substance was used. This step introduced an important difference with experiments already described (4). The bottle was kept in an incubator at 40° for 80–120 hr. Small samples of plasma were taken out every 24 hr for biological testing.

Purification procedures. Gel filtration. Only minor changes were introduced to the method described (4). The filtration was carried out with 40 ml of plasma samples on Sephadex G 200 (Pharmacia, Uppsala, Sweden) columns (120 × 2.4 and 85 × 3 cm) equilibrated with 0.5 *M* ammonium acetate buffer, pH 6.8. The flow rate was adjusted to 50 ml/hr and in each tube of the fraction collector, 8 ml of the effluent solution was collected. The presence of the pressor substance was investigated in every tube by injecting intravenously 0.1 to 0.2 ml of the solution in nephrectomized rats. As described below, only one peak of conspicuous pressor activity was found. The solutions having the highest activity corresponding to 4–5 tubes were pooled and freeze dried. The residue containing the pressor substance VA, after dialysis against distilled water or 0.9% saline solution, was ready for the experiments. Usually 0.1 ml of this solution containing 10 mg/ml was used.

Most of the pressor activity of the plasma incubated under strictly sterile conditions corresponds with the elution volume of the albumin after gel filtration in Sephadex G 100 (Fig. 1). The molecular weight of the active substance must be in the same range of the albumin molecule and confirms previous findings (13).

The pressor effect of the whole plasma or of its active fraction (VA) was compared with standard doses of angiotensin, bradykinin and adrenaline in several conditions: (a) in normal, nephrectomized and adrenalectomized rats; (b) before and after the injections of diverse adrenergic receptor blockers. In addition, the hypertension induced by slow infusion of the incubated plasma or the VA, was compared with that produced by the infusion of the nonincubated plasma, or of a 0.9% saline solution, or of a gelatin polymer as plasma expander (Haemacel, E. Boehring).

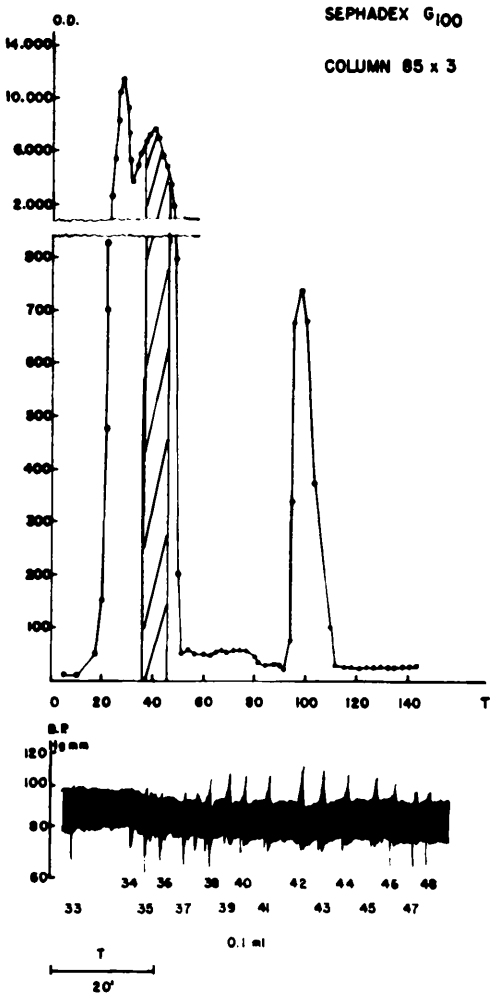

FIG. 1. (upper) Gel filtration through Sephadex G-100 of 40 ml of human plasma incubated 92 hr at 38°. The curve indicates optical density (OD) at 280 mμ wavelength; hatched zone correspond to the effluent solution containing the active substance; the values of the abscissa correspond to tube numbers; each tube contains 8 ml of the effluent solution. (lower) Blood pressure changes produced in a nephrectomized rat, by injections of 0.1 ml of the solution collected in the tubes whose numbers are indicated below.

Results. Incubation under sterile conditions of the heparinized plasma gives rise to a pressor activity, which progressively increases reaching its maximum after 80–96 hr (Fig. 2). This result confirms that heparin *in vitro* used in sufficient amount to prevent

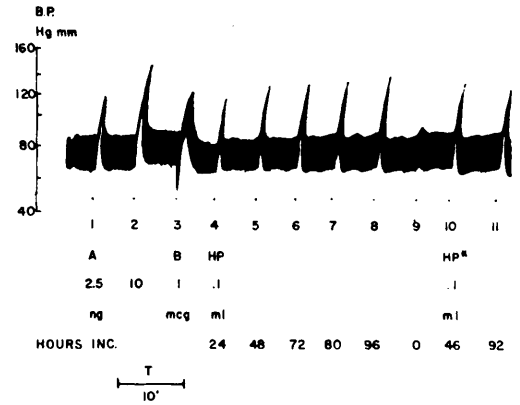

FIG. 2. Arterial blood pressure of a nephrectomized rat. Injections in the femoral vein: 1, 2) 2.5 and 10 ng of angiotensin II, respectively; 3) 1 μg of bradykinin; 4–9) 0.1 ml of the same human plasma (HP); 10, 11) 0.1 ml of plasma obtained from another person. Lowest row of values indicates hours of incubation for each plasma.

clotting (1 or 2 IU/ml) during incubation does not inhibit the appearance of the pressor activity. One-tenth ml of the incubated plasma of the 16 different persons gave a similar pressor effect equivalent to 5 $\pm$ 0.1 ng of valyl 5 angiotensin amide II. The infusion of 1 ml in 5 min produces a sustained increase of the blood pressure which at the end of the infusion goes down to the preinjection level (Fig. 3) or more frequently to a lower level. The hypertension cannot be ascribed to the fluid volume infused, because the nonincubated plasma produced either hypotension or insignificant increases. Plasma incubated for shorter periods gives proportionally lesser increases (Fig. 3). Similar activity as that exhibited by the incubated plasma was obtained with the active fraction VA, dissolved in 0.9% saline or Ringer solution. A similar volume of solvents does not modify significantly the pressure level (Fig. 4). After the infusion there is a constant tendency for a slow decline and then return to normal.

Effect of adrenalectomy. This operation performed immediately before the experiment does not suppress the pressor action either of the plasma or its active fraction. Insignificant differences in the magnitude of hypertension were observed between the nonadrenalectomized and adrenalectomized rats (Fig. 3). These results indicate that the adrenal gland is not required for the elevation of the blood pressure induced by the incubated plasma, whereas it reduces considerably or abolishes the pressor action of bradykinin (7).

Effect of the ganglionic blocking drug. During the marked hypotension induced by the azamethonium, the animals responded to adrenaline, angiotensin, incubated plasma and its VA with a greater blood pressure rise (Fig. 5); bradykinin in the blocked animals provokes biphasic or predominant pressor changes instead of hypotensive action (Figs. 5,6).

Effect of alpha receptor blocking drug. Phentolamine in doses ranging from 200 to 600 μg/100 g of body weight which reduced, abolished, or inverted the effects of adrenaline does not inhibit the rise of pressure achieved either by plasma or VA (Figs. 4, 5).

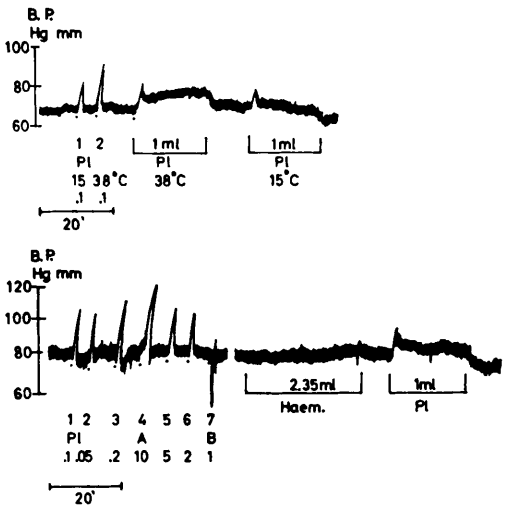

FIG. 3. (upper) Blood pressure changes of an adrenalectomized rat; injection in the femoral vein: 1, 2) 0.1 ml of human plasma incubated for 92 hr at 15 and 38°, respectively; further on 1 ml of the latter was infused for 20 min; finally 1 ml of the plasma incubated at 15° was infused at the same rate for 20 min. (lower) Blood pressure changes of a nephrectomized rat; injections in the femoral vein: 1, 2, and 3) 0.1, 0.05, and 0.2 ml of human plasma incubated for 96 hr at 38°, respectively; 4–5, and 6) 10.5 and 2 ng of angiotensin II; 7) 1 μg of bradykinin; later on 2.35 ml of Haemacel, and finally, 1 ml of the incubated plasma were infused.

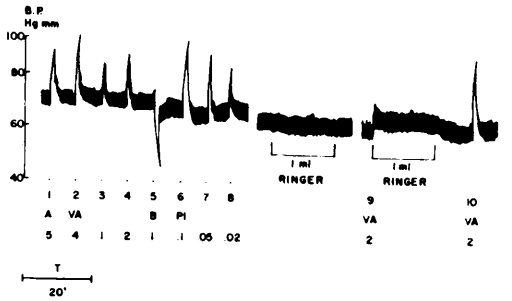

FIG. 4. Arterial blood pressure of a nephrectomized rat given with 400 μg of propranolol and 300 μg phentolamine; injections in the femoral vein: 1) 5 ng of angiotensin II; 2, 3, and 4) 1 and 2 mg of the active substance (VA) obtained from a plasma incubated for 96 hr at 38°; 5) 1 μg of bradykinin; 6, 7, and 8) 0.1, 0.05, and 0.02 ml of the incubated plasma; later 1 ml of Ringer solution; and 9) 1 ml of Ringer solution containing 2 mg of VA were infused; 10) 2 mg of VA dissolved in 0.1 ml of NaCl 0.9%.

In the same conditions the effect of angiotensin is considerably reduced and the pressor response to bradykinin disappears or is abolished (Fig. 6). Similar results were observed when propranolol injection preceded the administration of phentolamine (Fig. 5). These results give strong evidence that a liberation of adrenaline is not involved in the

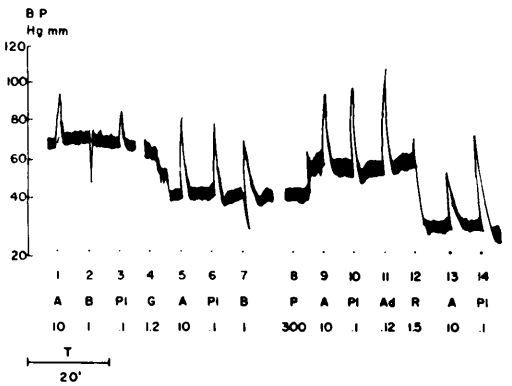

FIG. 5. Arterial blood pressure of a nephrectomized rat; injection in the femoral vein: 1) 10 ng of angiotensin; 2) 1 μg of bradykinin; 3) 0.1 ml of incubated human plasma (92 hr); 4) 1.2 mg of azamethonium; 5) 10 ng of angiotensin; 6) 0.1 ml of human plasma; 7) 1 μg of bradykinin; 8) 300 μg of propranolol; 9) 10 ng of angiotensin; 10) 0.1 ml of human plasma; 11) 0.12 μg of adrenaline 12) 1.5 mg of phentolamine; 13) 10 ng of angiotensin; and 14) 0.1 ml of human plasma.

pressor response which follows the injection of incubated plasma.

Effect of beta blocking drugs. After the injection of 150–250 μg/100 g of body weight of propranolol, the hypertensive reaction promoted by VA or plasma is significantly enhanced (Fig. 5). Only small changes in bradykinin action were observed and angiotensin did not vary its effect in a constant way. In some experiments the vasopressor effect was reduced and in others was not modified. The injection of azamethonium prior to propranolol does not modify the results, as compared to propranolol injected alone.

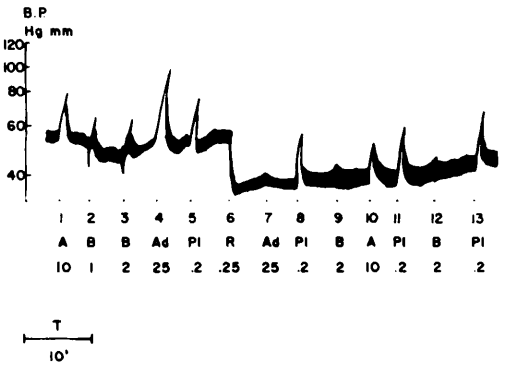

FIG. 6. Arterial blood pressure of a nephrectomized rat; injection in the femoral vein: 1) 10 ng of angiotensin II; 2 and 3) 1 and 2 μg of bradykinin, respectively; 4) 25 μg of adrenaline; 5) 0.2 ml of human plasma incubated 88 hr; 6) 0.250 μg of phentolamine; 7) 25 μg of adrenaline; 8) 0.2 ml of incubated human plasma; 9) 2 μg of bradykinin; 10) μg of adrenaline; 11) 0.2 ml of incubated human plasma; 12) 2 mg of bradykinin; 13) 0.2 ml of incubated human plasma.

Discussion. The failure of alpha and beta blocker drugs and adrenalectomy to prevent the hypertension produced by incubated plasma or VA rule out the possibility that this effect might be produced by the liberation of cathecholamines from adrenal medulla or from adrenergic neurones. Actually, after phentolamine or propranolol there is a tendency for a potentation of the pressor response to plasma, especially if the pressure level is low. It is a constant finding that when the blood pressure has been lowered by different procedures the rat responds with a greater rise in blood pressure. It is likely that

the more the vascular bed is expanded, the more is the range for contraction. But it is not excluded that the receptor blockers can facilitate the action of pressor substances contained in the incubated plasma through the inhibition of some buffer mechanism of the blood pressure regulation.

It is difficult to ascribe to a plasmakinin liberation the effect of incubated plasma. Either phentolamine or adrenalectomy inhibited the hypertensive phase of bradykinin and kallidin in the rat. (9)

The experiments do not provide data about the hemodynamic changes occurring under the influence of incubated plasma or its main pressor substance. Although it has been demonstrated that there is no change in the heart rate (2) and that presumably the hypertension is produced by a vasoconstriction, the mechanism of its action has not yet been established.

It is likely that the protein contained in the active fraction is a substrate or a carrier molecule able to liberate a polypeptide or the substance responsible for the hemodynamic changes as soon as the protein is incorporated in the recipient blood or reaches the blood vessels. An enzymatic system present in the rat could trigger the reaction.

According to Peterlik and Waldhäusl (13) the active pressor principle obtained from pig serum after 15 hr of incubation at 37° is due to a macromolecule similar in character to albumin. The hypothesis that this molecule can provide a vasoconstrictor substance is supported by the finding that albumin under pepsin hydrolysis releases a vasoconstrictor polypeptide having angiotensin-like activity (3).

Summary. In normal, nephrectomized or adrenalectomized rats the injection of azamethonium (ganglionic blocking agent) increases the pressor effect of incubated plasma or its active fraction (VA) and also that produced by angiotensin and bradykinin. However the pressor activity of incubated plasma or VA given by a single injection or infusion is: (a) not modified by adrenalectomy, and (b) not inhibited by the previous injections of α or β adrenergic receptor blocking agents, such as phentolamine and propranolol, respectively. These results rule out that the pressor effect is produced by the liberation of catecholamines. Since phentolamine alone or after azamethonium reduces the pressor effects of angiotensin and markedly inhibits or abolishes the pressor action of bradykinin, the conclusion can be drawn that the pressor effect of plasma and VA is not due to the release of angiotensin or bradykinin. The results of the gel filtration and electrophoresis of incubated plasma are in agreement with the view that VA is an albumin or an albumin bound molecule.

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